COMPARISON AND EVALUATION OF ANTIMICROBIAL EFFICACY OF CURCUMIN NANOPARTICLE HYDROGEL, CALCIUM HYDROXIDE PASTE AND 2% CHLORHEXIDINE GEL AS INTRACANAL MEDICAMENT AGAISNT ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

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MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

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CHENNAI

DECLARATION BY THE CANDIDATE

I hereby declare that the dissertation titled **"COMPARISON AND EVALUATION OF ANTIMICROBIAL EFFICACY OF CURCUMIN NANO PARTICLE HYDROGEL, CALCIUM HYDROXIDE PASTE AND 2% CHLORHEXIDINE GEL AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS-AN IN VITRO STUDY"**is a bonafide and genuine research work carried out by me under the guidance of **Dr. K. SENTHIL KUMAR, M.D.S,** Professor and Head, Department of Conservative Dentistry and Endodontics, Chettinad Dental College & Research Institute, Kelambakkam, Kanchipuram District.

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CERTIFICATE

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ABSTRACT

AIM:

To compare and evaluate antibacterial efficacy of Curcumin nano particle hydrogel, Calcium Hydroxide paste and 2% Chlorhexidine gel as intracanal medicament against Enterococcus faecalis.

MATERIALS AND METHODS:

Curcumin nano particles were prepared by ball milling technique after which it was prepared into a gel form using Polyethyleneglycol. Minimum inhibitory concentration and Minimum bacterial concentration of the prepared gel was analyzed. Thus based on the results 3% Curcumin nano particle hydrogel was tested in present study. 120 freshly extracted premolar teeth were decoronated and biomechanical preparation was done following normal irrigation protocol and divided into following groups: Group I-Normal saline, Group II Calcium Hydroxide paste [$Ca(OH)_2$], Group III Chlorhexidine gel (2% CHX) and Group IV 3% Nano curcumin gel (3% N Cu gel). The samples were inoculated with E.faecalis (ATCC) for 2 weeks after which the medicaments were placed inside the canal (n=27). After treatment dentin shaving was collected at end of day 1, 3 and 7 at varying depth of 200µm and 400µm in BHI broth. Colony forming units was counted with the help of digital colony counter.

RESULTS:

Group 3 (2%CHX) was the most effective against E.faecalis on all days. Group 2 $Ca(OH)_2$ and Group 4 (3%N Cu gel) was statistically significant from the control Group 1. Mean ± SD, $^{b}p<0.01$; $^{c}p<0.001$. On Day 1, significant difference is found between each group versus all other groups. On day 3, significant difference is found between each group versus all groups except between CHX and Nano Curcumin. On Day 7, significant difference is found between each group versus all the groups

CONCLUSION:

Nano Curcumin gel can be used as an Intracanal medicament as the efficacy is almost similar to commercially available intracanal medicaments.

KEYWORDS:

Curcumin nano particle hydrogel, 2%Chlorhedine gel, Calcium Hydroxide paste, Intracanal medicaments and E.Faecalis.

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LIST OF ABBREVIATIONS

ABBREVIATION	WORD EXPLANATION
ATCC	American type culture collection
ANOVA	Analysis Of Variance
BHI	Brain Heart Infusion
$Ca(OH)_2$	Calcium Hydroxide
CFU	Colony Forming Units
CHX	Chlorhexidine Gluconate
N Cu Gel	Nano Curcumin gel
E.faecalis	Enterococcus Faecalis
EDTA	Ethylene Diamine Tetra acetic Acid
H_2O_2	Hydrogen Peroxide
p Value	Probability Value
PH	Potential of Hydrogen
NaOCl	Sodium Hypochlorite
TSBG	Tryptic Soy broth with 0.5% Glucose
g	Dimethyl sulfoxide
MHI	Mueler Hinton broth
GG	Gates Glidden
FTIR	Fourier transform Infrared Spectroscopy
PEG	Polyethylene Glycol
MIC	Minimum Inhibitory concentration
MBC	Minimum Bactericidal Concentration

SD	Standard Deviation
FtsZ	Filamenting temperature sensitive mutant Z
SPSS	Statistical package of social science
ZOI	Zone of inhibition

INTRODUCTION

Microorganisms play a key role in development of pulp and periapical diseases[1]. It is always a complicated task to eliminate microorganism from the root canal owing to its complex anatomy[2]. Various techniques and measure have been taken to eliminate micro organism from the root canal including use of various instrumentation technique, irrigation regimens and intracanal medicaments[3]. Literature suggests that mechanical instrumentation alone cannot eliminate the bacteria in root canal system, hence it is recommended to use irrigants and intracanal medicaments to eliminate microorganisms. [4]

In failed endodontic cases, Enterococcus faecalis (E.faecalis) is the predominant organism. E.faecalis is gram-positive cocci that occur in pairs or short chains, it is a facultative anaerobe present in small proportion[6]. It is predominant flora in persistent endodontic infection and its role in endodontic failure is mainly due to its ability to invade dentinal tubules, persist in harsh environment including high alkaline pH, salt concentration, resists bile salts and can survive temperature upto 60^0C[6]. E.faecalis decreases the host response and action of lymphocytes. It can colonize in root canal and survive without support of other bacteria[7]. In Primary Endodontic infection prevalence of E.faecalis is 40 % and in cases of persistent Endodontic infection it ranges from 24-77%.[8]

Various intracanal medicaments have been introduced in dentistry to eliminate E.faecalis from endodontic environment. Some of the most commonly used endodontic medicaments include Calcium hydroxide, Chlorhexidine digluconate gel, MTAD, Triple antibiotic paste[9]. Recently herbal medicaments have proven to be effective against E.faecalis. Liquorice extract, Propolis, Morindacitrifolia, Ethyl acetate extract of Arctium lappa plants and Curcumin have been used against E.faecalis[10,11].

Calcium hydroxide has been in practice for over a century, since its introduction into dentistry in early 1920s by Hermann were he used calcium hydroxide as intracanal medicament. It promotes healing and is a potent bactericidal [12] .

Chlorhexidine is a base and stable as a salt. The most common oral formulation is 0.2 % Chlorhexidine gluconate, which is used as a mouth rinse and at physiological pH, it readily dissociates and releases the positively charged Chlorhexidine component[13]. At low concentration (0.2%) CHX is bacteriostatic and at higher concentration (2%), CHX is bactericidal as there is precipitation of bacterial cell cytoplasmic contents resulting in cell death[14]. Another advantage of CHX is its ability to be retained in dental hard tissues, which is gradually released into oral fluids for hours and this is known as substantivity[15.]

Intracanal medicaments from nature

Recently natural and herbal products have played a key role in endodontics, they are known to have strong antimicrobial action against most of the endodontic flora[16]. Use of plant products in medicine is known as phytomedicine or phototherapy[10]. As chemical and synthetic products are expensive and cause cytotoxic reactions in the oral cavity, herbal products can be used as an alternative and they are less toxic[17].

CURCUMIN:

Turmeric has anti inflammatory, antioxidant, antiviral and antimicrobial properties. Curcumin is naturally occurring chemical compound found in turmeric, it is used as antiseptic, antidiabetic, and immunomodulatory functions. Curcumin can be used as a pain killer due to its anti inflammatory properties and also promotes wound healing[18]. Recently Curcumin is used as antimicrobial agent in infected root canals. The main advantage of Curcumin is that it is readily available and cheap. Several studies have shown that Curcumin is effective against most of the endodontic pathogens[19]. Turmeric gel 2% is used as a local drug delivery in the field of periodontics and was effective in reducing the pocket depth[19,20].

Several In vitro studies done by Kumar et al[21], Hegde et al[22] have shown that turmeric extract has significant antimicrobial efficacy against E.faecalis and can be used in field of Endodontics.

Nano curcumin is readily dispersible in water compared to Curcumin and aqueous dispersion of Nano curcumin was more effective against microorganisms. Nano curcumin has particle size ranging from 30-50nm, where as conventional Curcumin had particle size of 200nm[23].

AIMS AND OBJECTIVES

AIM

To compare and evaluate the antimicrobial efficacy of three intracanal medicament against E.faecalis

OBJECTIVE

To compare and evaluate the antimicrobial efficacy of Curcumin nanoparticle hydrogel, Calcium Hydroxide paste and 2%Chlorhexidine gel as intracanal medicament against E.faecalis by checking for colony forming units after a period of 1,3 and 7 days.

NULL HYPOTHESIS

There is no significant difference among the various intracanal medicaments used against E.faecalis

REVIEW OF LITERATURE

U.Sjogren et al[24] **in 1991** did a study on 30 extracted teeth which were necrotic or had a periapical pathology and divided it into two groups in which 18 teeth had Calcium hydroxide dressing for 7 days while remaining 12 teeth had dressing for 10min. It was found that intracanal medicament placed for 7 days eliminated bacteria effectively while the 10min dressing was ineffective.

I.Heling et al[25] **in 1992** evaluated the effect of 0.2% Chlorhexidine gluconate solution, Chlorhexidine in sustained release vehicle and Camphorated paramonochlorphenol against E.faecalis. After preparing the dentin blocks it was contaminated with E.faecalis for 3 weeks. The medicaments were placed into the root canal and antibacterial efficacy was checked at intervals of 24h, 48h and 7 days by collecting the dentin shavings in growth medium. Optical density was recorded with the help of spectrophotometer and it was found that significant difference was found between experimental group and control group.

E.Kontakiotis et al[26] **in 1995** did an in vitro study on antimicrobial efficacy of Calcium hydroxide against predominant micro flora in root canal by vapour release. 20 obligate and facultative anaerobes were incubated in anaerobic chamber for 7 days. Experimental group composed of one plate with bacterial species and other plate

containing 32 gm of calcium hydroxide mixed in a ratio of 6:4 and incubated for 72hours. Control group is composed of only bacterial species. It was found that Calcium hydroxide absorbed carbon dioxide and exerts antimicrobial effect.

Siqueira et al[27] **in 1997** evaluated the antimicrobial efficacy of Chlorhexidine gel, Calcium hydroxide + distilled water, Calcium hydroxide + Camphorated paramonochlorphenol and 10% Metronidazole gel against anaerobic micro flora. Agar diffusion test revealed that Calcium hydroxide and paramonochlorphenol combination was effective in eliminating the microorganisms compared to other groups.

Love[28] **in 2001** did a study to find out survival of E.faecalis in dentinal tubules and reinfect the teeth after obturation. The organisms used were S.mutans, S.gordonii and E.faecalis. Its ability to invade dentinal tubules and bind to Type I collagen in presence of human serum was tested by dentin invation and micro well titre. Results showed that S.mutans and S.gordonii did not adhere to collagen but E.Faecalis was able to adhere and invade dentinal tubules.

Estrela et al[29] **in 2001** used different vehicles of Calcium hydroxide and tested its antimicrobial efficacy against endodontic micro flora. A combination of $Ca(OH)_2$+ saline, $Ca(OH)_2$+ 1% CHX solution,

$Ca(OH)_2$+ Camphorated parachlorophenol, $Ca(OH)_2$+ 3% Sodium lauryl sulphate and $Ca(OH)_2$+ Otosporin were used. 50 sterile paper points was inoculated in the bacterial medium for 3min after which it was placed in direct contact with medicaments. The paper points were removed and placed in BHI broth and turbidity of the culture medium was evaluated. It was found that antimicrobial effect occurred after 48hours irrespective of the vehicle used.

Evans et al[30] **in 2002** used pretreated Calcium hydroxide with de-ionized water, resultant Calcium hydroxide had a pH of 11.5. Additional Calcium hydroxide solution was prepared by diluting stock solution in deionized water, so solutions of pH 11.1 and 10.3 were obtained. It was found that E.faecalis was resistant to Calcium hydroxide at pH 11.1 and 10.3 due to proton pump mechanism, which lowers the internal pH of the cell and maintains it, whereas at high pH 11.5 it did not survive as the adaptive mechanism resulted in acidic pH within the cell, which damaged the cytoplasm.

Gomes et al[31] **in 2003** evaluated the efficacy CHX gel and calcium hydroxide combined together against E.faecalis in extracted bovine teeth. It was found that combination of 2% CHX gel and $Ca(OH)_2$ inhibited growth on 1st and 3rd day but efficacy reduced on 7Th and 15Th day compared to Calcium hydroxide, which inhibited growth on all days.

Nageshwar et al[32]in 2004 tested the efficacy of Calcium hydroxide and 2%CHX gel, Calcium hydroxide and saline, against E.faecalis. 30 Central incisors inoculated with E.faecalis for 3 weeks after which the medicaments were placed inside the canal with the help of lentulosprial. After 7days the dentin shavings were collected with the help of round bur and spread on BHI agar. CFU showed that Calcium hydroxide and 2%CHX was more effective compared to Calcium hydroxide and saline combination.

Kwon et al[33]in 2004 evaluated the vibrational modes of molecules of Calcium hydroxide using FT Raman spectroscopy. According to this study Calcium hydroxide was converted into Calcium carbonate in apical region alone within 2 days. It was found that 11% of Calcium hydroxide was converted into Calcium carbonate. The intensity of conversion was rapid in first 2days and after that it gradually reduced. 90% of Calcium hydroxide remained unchanged. It was found that $Ca(OH)_2$, as an intracanal medicament does not lose activity within a short period and may allow for longer inter-visit periods in endodontic therapy.

Schafer and Bossmann[34]in 2005 studied the effectiveness of 2%CHX gel, $Ca(OH)_2$ paste and 2% CHX +$Ca(OH)_2$, as intracanal medicament in extracted teeth against E.faecalis. 60 Extracted single-rooted human teeth were instrumented up to size 40 using k-file. After removal of the smear layer, E.faecalis was inoculated

into the root canal and incubated. After incubation, the inoculum was removed and the root canals were filled with the following medicaments $Ca(OH)_2$ paste, CHX 2%, and a mixture of CHX and $Ca(OH)_2$ paste. The teeth were then incubated for 3 days. After incubation, medicaments were washed with saline and dentin shavings were collected. It was found that CHX was significantly more effective against E.faecalis than $Ca(OH)_2$ paste or a mixture of CHX with $Ca(OH)_2$ paste. There was no increase in the efficacy of $Ca(OH)_2$ paste when CHX was added. Thus the results stated that 2%CHX gel is effective in elimination of E.faecalis from dentinal tubules.

Sedgley et al[35] **in 2005** did a study on long term survival of E.faecalis in root canal filled teeth. 150 single rooted teeth were instrumented upto 50k file and were divided into 6 groups. In each group, 10 samples were inoculated with gelatinase- producing E. faecalis OG1-S and the other 10 with its gelatinase-defective mutant E. faecalis OG1-x and remaining 5 uninoculated. The root canals in-group 1 and 2 were inoculated with 10^6 bacteria and incubated for 48hours at 37 °C after which the teeth were obturated. Groups 3-6 were inoculated with 10^6, 10^5, 10^4 and 10^3 bacteria and left unfilled. Group 1,3-6 was analyzed after 6month and group 2 was analyzed after 12months. It was found that viable E.faecalis was present in all root canal filled teeth and its presence was ranging from 95-100% in unfilled teeth. Thus clinical implication is that

viable E.faecalis present at time of root canal treatment could be the source of reinfection.

Abdullah et al[36] **in 2005** did a study to investigate and compare the efficacy of Calcium hydroxide (pH 12.3), 0.2% Chlorhexidine gluconate, 17% Ethylene-diamine-tetra-acetic acid, 10% Povidone iodine and 3.0% Sodium hypochlorite (NaOCl) as a root canal irrigants on isolate of E.faecalis grown as biofilm or planktonic suspension phenotype. The time period ranged from 1,2,4,8,15,30 and 60minutes. The results of this study showed that it is hard to eliminate E.faecalis in biofilms than in planktonic suspension if the antimicrobial agents do not possess any organic tissue dissolving properties. The antimicrobial effectiveness of test agents was dependent on bacterial phenotype, antimicrobial agent and duration of contact with the agent. NaOCl for 2min was most effective agent against E.faecalis.

Kayaoglu et al[37] **in 2005** evaluated the growth of E.faecalis at different pH levels from 7.1-9.5 and its adherence to Bovine serum albumin (BSA) and Collagen type I. E.faecalis strains was grown in broth of adjusted pH varying between 7.1-9.5.wells were coated with BSA and type I collagen and small aliquots of bacteria was added into the wells. Bacteria adhering to the surfaces were stained with crystal violet. Spectrophotometric measurements of the dissolved stain were used to assess the number of bacteria adhering

to the surfaces. pH 7.1-grown bacteria bound to BSA significantly more than the other BSA groups. On the contrary, the adhesion to collagen type I-coated surfaces of bacteria grown at pH 8.0 and 8.5 was significantly greater than for those grown at pH 7.1

Ercan et al[38] **in 2006** did a study to comparatively evaluate antimicrobial efficacy of 2%CHX gel, $Ca(OH)_2$ +2% CHX, $Ca(OH)_2$ against E.faecalis and candida albicans. 80 freshly extracted teeth was cleaned and shaped upto 50-size K file. After tooth preparation samples were inoculated with E.faecalis and candida albicans for 3 weeks. The samples were removed and placed with the medicaments, after 7,15 and 30 days the dentin shavings were removed antimicrobial efficacy was assessed by means of CFU, the results showed that 2%CHX gel was more effective than all other groups.

Yang et al[39] **in 2006** did an In vitro study to determine the effects of smear layer and chlorhexidine (CHX) treatment on the adhesion of Enterococcus faecalis to bovine dentin. Forty bovine incisors were prepared and divided into 4 groups of 10 each. The blocks in group 1 were placed in sterile saline for 5 minutes, group 2 were treated with 17% EDTA for 5 minutes, group 3 were placed in 2% CHX for 7 days and group 4 were treated with 17% EDTA for 5 minutes, and then placed in 2% CHX for 7 days. All the blocks were immersed in a suspension of E. faecalis for 3 hours. The bacteria

adhering to the dentin surface were counted by examination using a scanning electron microscope. The results showed that EDTA and CHX combination showed least adhesion of bacteria to dentin surface.[39.]

Krithikadatta et al[40] in 2007 evaluated the efficacy of 2% CHX gel, 2% metronidazole gel and bioactive glass when compared with $Ca(OH)_2$ as intracanal medicament against E.Faecalis. Samples were prepared and inoculated with bacteria for 3 weeks. After which the medicaments were placed and antimicrobial action was checked at the end of 1,3, and 7 days by means culture method. It was found that CHX was most effective against E.faecalis compared to other groups.

Ballal et al[41] in 2007 evaluated antimicrobial efficacy of Calcium hydroxide, 2% CHX gel and their combination against Candida albicans and E.faecalis. Inoculate of these organisms were used to make lawn cultures on Sabouraud's dextrose agar and blood agar plates. Wells were prepared with these lawn cultures and filled with Calcium hydroxide paste, 2% Chlorhexidine gel and their combination. The agar plates were kept overnight for incubation at 37℃ and the zone of inhibition was examined after 24 and 72 hours. It was found that 2% CHX gel was more effective in eliminating Candida albicans and E.faecalis.

Blanscet et al[42] **in 2008** did a study to determine varying percentage of aqueous Calcium hydroxide or using different vehicles had antibacterial effect against endodontic pathogen. Mixtures of Calcium hydroxide powder and sterile saline in 40, 50 and 60 percent concentrations and the commercial preparations Ultra Cal XS and Vitapex with aqueous Methylcellulose were tested against six known endodontic pathogens with an agar diffusion method. Each medicament was placed in one of five wells, in each of 10 agar plates for cultures of each bacterial species. Zones of inhibition were measured after 48 hours incubation for aerobes and 96 hours incubation for anaerobes. High concentration of calcium hydroxide showed maximum zone of inhibition. Ultra Cal XS was effective compared to Vitapex. 35% Uracil XS was effective as 50% Calcium hydroxide preparation.

Chivataranukul et al[43] **in 2008** investigated the invasion of dentinal tubules by E.faecalis. 16 single rooted premolar were divided into 2 groups of 8 each. The teeth samples were instrumented and in one group smear layer was left intact while in other group it was completely removed. The teeth samples were split using diamond saw to expose the fractured orthodentin and aligned dentinal tubules. This sample was inoculated with E.faecalis. In the adhesion studies, significantly more bacteria adhered to fractured OD than to dentinal tubule walls. With respect to the tubule wall, adherence was greater in inner versus outer

dentine and greater when bacterial adhesion was tested in chemically defined medium than in Phosphate-buffered saline.

Lee et al[44] in 2009 did an in vitro study to determine if CHX reduced the inflammatory activity of Enterococcus faecalis and its major virulence factor, lipoteichoic acid (LTA). An Enzyme- linked immunosorbent assay (ELISA) showed that CHX-killed E. faecalis was less potent than heat-killed E. faecalis in the production of tumor necrosis factor (TNF alpha) by a Murine macrophage cell line. Interestingly, pretreatment of LTA with 2% CHX for 6 hours or with 0.2% CHX for 24 hours almost eliminated the TNF alpha. They suggested that CHX could inactivate LTA of E.faecalis leading to the reduction in inflammatory responses.

Turk et al[45] in 2009 evaluated the antimicrobial activity of $Ca(OH)_2$ +Glycerin, $Ca(OH)_2$,CHX + Cetrimide, or distilled water against E.faecalis and C.albicans by agar diffusion method. It was concluded that the antimicrobial activity of $Ca(OH)_2$ with glycerin was not effective in eradicating the microorganism compared to CH and CHX or cetrimide combination[45]

Estrela et al[46] in 2009 reviewed antimicrobial efficacy of intracanal medicaments on bacterial biofilm. 91 articles were selected, of which 17 (18.7%) were literature reviews, 8 (8.8%) referred to *in*

vivo studies (7 in humans and 1 in animals), 19 (20.1%) referred to *in vitro* studies, 7 (7.7%) articles of biofilm in membrane filter models and 12 (13.1%) articles on root dentin biofilm models, 9 (10%) were studies on non endodontic biofilm, and 33 (36.2%) articles related to other types of study. None of the articles met the inclusion criteria and results of analysis showed that the intracanal medicaments did not have sufficient effect of bacterial biofilm.

Rasimick et al[47] in 2010 evaluated the substantivity effect of 2%CHX and 3% Doxycycline, which was taken up into root dentin by using reverse phase high performance liquid chromatography. After a day the dentinal shavings containing active medicaments were removed and it was decalcified. The remaining amount of Doxycycline and CHX were measured, it was concluded that CHX was more stable in root canal than Doxycycline. Half life of doxycycline and CHX was 3 weeks and 14 weeks respectively.

Delgado et al[48] in 2010 tested antimicrobial efficacy of 2% CHX alone or in combination with $Ca(OH)_2$ against E.faecalis. Human uniradicular teeth were contaminated with E.faecalis for 3 weeks. Intracanal medicaments were placed for 14 days, after which dentin shavings were obtained at depth of 0 to100 µm and 100 to 200 µm. CFU count revealed that CHX had high antibacterial effect compared to $Ca(OH)_2$. They found that E.faecalis was still present

in the dentinal tubules after 14 days but it was in a non culturable state.

Rocas and Siqueira[49] **in 2011** did a study on 24 necrotic canals, where pre instrumentation sample was taken with the help of sterile paper points (S1), another sample taken after chemecomechanical preparation (S2) and after 7 days calcium hydroxide in either glycerin or camphorated paramonochlorophenol placed sample was taken as (S3). Bacterial, archaeal, and fungal presence was evaluated by polymerase chain reaction (PCR). All S1 samples were positive for bacteria but negative for both archaea and fungi. Over all, 46% of S2 samples and 62.5% of S3 samples were PCR-negative for bacteria. Specifically, S2 and S3 samples yielded negative PCR results in 50% and 58% of the canals in the CHG group and in 42% and 67% of the canals in the CHPG group, respectively.

Signoretti et al[50] **in 2011** evaluated the influence of 2% CHX gel and Calcium hydroxide, on pH, calcium release and release of endotoxins. Calcium release was verified by Atomic-Absorbance Spectrophotometry, and pH was measured with a pH meter. For endotoxin quantification, extracted human teeth previously contaminated with standard endotoxin were filled with: group I- $Ca(OH)_2$+ saline, group II- CHX and group III- $Ca(OH)_2$, CHX for 14 days. It was found that group I and group II had alkaline pH at all times. Group II and III showed significantly reduced release of

endotoxins. Thus the study concluded that CHX did not interfere with chemical composition of Calcium hydroxide, it infact improved the properties of Calcium hydroxide.

Vaghela DJ et al[51] in 2011 did a study to investigate the antimicrobial efficacy of $Ca(OH)_2$ with propylene glycol, $Ca(OH)_2$ with iodoform in silicone oil and compared with 2% CHX gel against E.faecalis and C.albicans. The efficacy was assessed using dentin tubule model at depth of 200 µm and 400 µm in uniradicular teeth. It was found that $Ca(OH)_2$ with propylene glycol was most effective against E.faecalis whereas $Ca(OH)_2$ with iodoform in silicone oil was effective against C.Albicans. 2% CHX was effective against both species.

Silva et al[52] in 2011 did a study and evaluated cleaning of dentinal wall after removal of different Calcium hydroxide pastes. Sixty-eight single-rooted teeth were prepared using the step-back technique and randomly divided into 4 groups according to medication used: $Ca(OH)_2$with 0.2% chlorhexidine solution (Group 1), $Ca(OH)_2$ with propylene glycol (Group 2), $Ca(OH)_2$ with antibiotic paste (ciprofloxacin, metronidazole) and distilled water (Group 3), and $Ca(OH)_2$ with antibiotic paste and propylene glycol (Group 4). The samples were stored at 37°C and 100% relative humidity for 21 days. The medicaments were removed using 5 ml 1% NaOCl, instrumentation with master apical file, 5 ml 1% NaOCl,

patency with the K-file #10, ultrasonic instrumentation, and 10 ml 17% EDTA. The specimens were analyzed using Scanning Electron Microscopy and chemical analysis. It was found that all the groups of $Ca(OH)_2$ are blocking dentinal tubules regardless of the vehicle used[52].

Bhawana et al[23] 2011 developed a method for the preparation of nanoparticles of Curcumin with a view to improve its aqueous-phase solubility and examine the effect on its antimicrobial properties. Nanoparticles of curcumin (nanocurcumin) were prepared by a process based on a wet-milling technique and were found to have a narrow particle size distribution in the range of 2- 40 nm. The results demonstrated that the water solubility and antimicrobial activity of curcumin markedly improved by particle size reduction up to the nano range.

Souza et al[53] in 2012 did a study on 45 freshly extracted human teeth and checked the susbstantivity of CHX in different formulation. Three groups of 15 each were taken group I-2%CHX solution, group II -2% CHX gel and saline group. The substantivity was checked using reverse-phase high-performance liquid chromatography. The substantivity was checked at intervals of 24 hours, 30 days and 90 days and it was found that some amount of CHX was retained in root dentin for upto 90 days.[53]

Baca et al[54] **in 2012** did an in vitro study to evaluate the antimicrobial substantivity of chlorhexidine (CHX) and cetrimide (CTR) against Enterococcus faecalis in dentin- volumetric unit exposed for 1 minute. Dentin blocks of human molars, with and without collagen, were treated for 1 minute with 0.2% and 2% CHX and 0.2% CTR. Afterwards, they were exposed to E. faecalis suspension to determine the antimicrobial substantivity over a period of 60 days. The present study shows that 2% CHX used for 1 minute provides the longest substantivity followed by 0.2%.

Alireza Adl et al[55] **in 2012** determined the Antimicrobial ability of Triple antibiotic paste and its components along with Calcium hydroxide against E.faecalis. Medicaments were divided into 9 groups; Triple antibiotic powder with saline or chlorhexidine, Metronidazole, Ciprofloxacin, Minocycline antibiotics were also separately tested (with normal saline), and $Ca(OH)_2$-(with saline or CHX). An agar well diffusion assay and MIC method were used to determine the efficacy of the experimental medicaments in removing E. faecalis. The largest inhibition zones were observed for the triple antibiotic mixture/saline, triple antibiotic mixture/2% chlorhexidine and minocycline/saline, and the smallest for $Ca(OH)_2$ (with saline or CHX).

Atila-Pektas et al[3] **in 2013** compared the antimicrobial efficacy of $Ca(OH)_2$, $Ca(OH)_2$Plus Point (medicated gutta-percha with

$Ca(OH)_2$), Activ Point (medicated gutta-percha with CHX diacetate), 1% CHX gluconate gel and bioactive glass (S53P4), against E.faecalis and S.mutans. 120 uniradicular teeth were taken for this study and dentin blocks were prepared. The blocks were contaminated with E.faecalis and incubated for 3 weeks. The medicaments were placed inside the canal and after 7 days samples were taken with the help of sterile paper points and CFU was estimated. The results showed that CHX was most effective compared to the medicaments containing calcium hydroxide.

Neelakantan et al[56] **2013** evaluated the antimicrobial efficacy of Curcumin, Sodium hypochlorite (NaOCl) and Chlorhexidine (CHX) against Enterococcus faecalis biofilm. 96 extracted human teeth were taken and instrumented, E.faecalis biofilm was inoculated in internal aspect of tooth. At the end of the 2nd day, 2nd week and 8th week, specimens were treated for 30 min with one of the test solutions or saline (control) and the surviving colony-forming units (CFU/mL) were recorded. Results showed that only NaOCl showed complete eradication of bacteria at all time periods. In the 2-day and 2nd week biofilms, Curcumin and NaOCl were effective against E. faecalis biofilm and showed complete inhibition. Curcumin was found to be more effective than CHX. However by the end of 8Th week the efficacy of curcumin reduced and was very low compared to CHX.

Mandroli and bhat et al[57] **in 2013** investigated the anti-microbial potential of Curcumin, against standard strains of common endodontic bacteria. The bacterial strains of Streptococcus mutans, Actinomyces viscosus, Lactobacillus casei, Porphyromonas gingivalis, Prevotella intermedia and Enterococcus faecalis were used. The colonies were transferred into sterile Brain heart infusion broth to which serially diluted Curcumin samples were added. The tubes were then incubated for 24 hours at 37º C. The last tube with clear medium was considered to be without any growth and taken as MIC value. Mean MIC values of Curcumin showed that it has the potential to be developed into medicament for the treatment of various endodontic diseases.

Taur et al[58] **2013** evaluated the antimicrobial efficacy of Turmeric extract, Calcium hydroxide and 2% CHX gel against E.faecalis and also tested the micro hardness of various medicaments on root dentin. 120 Dentin blocks were prepared and antimicrobial efficacy was tested, 20 blocks were subjected to Vickers hardness indentation machine to check the micro hardness after 24 hours of placing the medicaments. Results showed that, CHX showed the best antimicrobial efficacy against E.faecalis compared to other groups and Calcium hydroxide had highest effect on micro hardness of dentin whereas turmeric and CHX did not have any effect[58].

Mohammed Ali et al[59] **in 2014** did an in vitro study to evaluate the antimicrobial activity of Calcium hydroxide (CH), 2% Chlorhexidine gel (CHX), Triple antibiotic paste (TAP) and Nano silver (NS) on E.faecalis. Fifty extracted single rooted teeth were taken and inoculated with E.faecalis for 3weeks. The medicaments were placed and microbial samples were obtained after 7 days and optical density of the cultures was determined after 24 h of incubation. The results showed that CHX gel and TAP were significantly more effective against E.faecalis than CH, which was also significantly more efficient than NS and normal saline. However NS gel was not efficient enough against E.faecalis, TAP and CHX gel showed better antibacterial efficacy than CH

Jaheer et al[60] **in 2014** did a study to analyze the sustained release of intracanal medicaments with or without a carrier and tested their antimicrobial efficacy against Candida albicans and Enterococcus faecalis. 80 single rooted anterior teeth were selected, dentin blocks were prepared and one half of the samples were contaminated with E.faecalis and the other half with C.Albicans. The samples were further divided into four test groups TAP + saline, Chitosan + TAP, $Ca(OH)_2$+ saline and $Ca(OH)_2$+ Chitosan. Chitosan was used as vehicle for Triple antibiotic paste (TAP) and Calcium hydroxide. Antimicrobial assessment was performed on second and seventh day. Dentin samples were collected after each time intervals and the number of colony-forming units (CFUs) was determined. All the

medicaments showed effective anti bacterial and anti fungal properties. Group II and group IV were effective and significantly better than other groups.

Chamele and bhat et al[61] **in 2014** evaluated the antimicrobial efficacy of Turmeric extract, Calcium hydroxide paste and saline against E.faecalis in deciduous teeth. 60 unirooted deciduous teeth were prepared and inoculated with E.faecalis for 2 weeks. After inoculation, the teeth samples were subjected to the above-mentioned medicaments. At the end of 7 days the dentin shavings were removed and collected in culture medium. CFU were counted with the help of colony counter. Results showed that turmeric extract is effective against E. faecalis. Calcium hydroxide and Curcumin showed better antibacterial effect. There is 50% reduction in colony count was observed when treated with Curcumin. Hence there is possibility of use of $Ca(OH)_2$, which can be substituted with Curcumin as an intracanal medicament.

Marickar et al[62] **2014** compared antibacterial activity of Propolis (30% in methyl cellulose), Curcumin (2.5mg/ mL of methyl cellulose), 2% Chlorhexidine gel (CHX), 2% Metronidazole gel (MZ) and a mixture of 2% CHX and 2% MZ against Enterococcus faecalis. Agar diffusion test and tube dilution test showed that CHX and turmeric extract showed the best antimicrobial activity against E.faecalis compared to Calcium hydroxide.

D.A. Attia et al[63]in 2015 conducted an in vitro study, to compare the antimicrobial effect of $Ca(OH)_2$ paste, CHX gel and Antibiotic-Corticosteroid paste against S. mutans, E.faecalis and C.albicans. Eighty four single rooted extracted human teeth with straight root canals were selected, All canals were prepared up to size 40 master apical file under irrigation with NaOCl solution. Roots were sterilized, mixed suspension of the three type's of microorganisms were inoculated into the root canal and incubated at 37^0C for 14 days. The roots were divided into 4 equal groups according to the intracanal medications used- $Ca(OH)_2$, CHX, Antibiotic-Corticosteroid paste and saline. Each main group was further subdivided into 3 according to the isolated organism. Subgroup (A): S.mutans, Subgroup (B): E.faecalis and Subgroup(C): C.albicans. The medicated roots were incubated for 7 days at 37^0C and irrigated to remove the medications. Samples were taken from root canal lumen and CFU were counted. CHX gel showed the best effect against all tested microorganisms at both experimental sites, while Antibiotic-Corticosteroid paste was the least effective and also found that S.mutans was the most sensitive microorganism to the whole tested medications, while C.albicans was the most resistant.

Mozayeni et al[59] in 2015 compared the antifungal effect of $Ca(OH)_2$, 2% CHX and Nanosilver gels on C.albicans. Eighty-one single-rooted teeth were selected. After root canal preparation, the teeth were contaminated and were randomly divided into 4 groups.

In experimental groups, 24 teeth were selected and completely filled with $Ca(OH)_2$, 2% CHX and Nano silver gels in each group. Nine teeth were selected in the control group and filled with saline solution. After 1, 3, and 7 days, samples were obtained using 30 size sterile paper points, and #2 and #4 Gates Glidden drills and cultured on solid Sabouraud agar. The results demonstrated that $Ca(OH)_2$and 2% CHX had equal antifungal effects on samples taken by paper point and #2 Gates Glidden drill at all time points. Both $Ca(OH)_2$and 2% CHX were more effective than Nano silver at all time periods. There was no statistically significant difference between medicaments in samples taken by #4 Gates Glidden drill. $Ca(OH)_2$ and 2% CHX gels have significantly higher antifungal activity than Nano silver gel. $Ca(OH)_2$ and 2% CHX gels were also equally effective against C.albicans.

Saha et al[64] **2015** evaluated the effectiveness of Curcuma Longa, Propolis, Metronidazole with CHX gel and Calcium hydroxide against E.Faecalis. The Optical density value showed that all groups were effective against E.faecalis, Propolis was better compared to other intracanal medicaments.

Sharmila et al[65] **in 2016** evaluated the efficacy of 5 intracanal medicaments Light activated Curcumin, Triple antibiotic paste (TAP), Double antibiotic paste (DAP), Chlorhexidine and Calcium hydroxide against mature biofilms of E.faecalis. Confocal

microscopy was used to analyze the biofilm mass and percentage of live/dead bacteria within the root canal as well as dentinal tubules. Dentinal shavings obtained from the root canal walls (at 200 and 400 microns depth) were used to quantify the colony forming units/mL. The results showed that Light activated curcumin and Triple antibiotic paste brought about complete disruption of the biofilm structure while Chlorhexidine and Calcium hydroxide were not significantly different from the control. Light activated curcumin brought about the highest percentage of dead cells at both depths, but this was not significantly different from Triple antibiotic paste. Curcumin, TAP and DAP brought about a significant reduction of CFU/mL at both depths compared to the control and other groups Light activated curcumin brought about significant reduction of bacteria at both depths.

Shrestha and Kishenin[66.] **2016** reviewed the Application of Antibacterial Nanoparticles in Endodontics. Nanoparticles have enhanced physicochemical properties, such as ultra small sizes, large surface area/ mass ratio, and increased chemical reactivity as a result of which the treatment of dental infections has significantly improved .The article did a comprehensive review on the scientific knowledge that is available on the application of antibacterial nanoparticles in endodontics. The application of nanoparticles in the form of solutions for irrigation, medication, and an additive within sealers/restorative materials has been evaluated to primarily

improve the anti biofilm efficacy in root canal and restorative treatments. In addition, antibiotic or photosensitizer functionalized nanoparticles have been developed recently to provide more potent antibacterial efficacy.

Zancan et al[67] **in 2016** investigated the pH, calcium release, solubility, and antimicrobial action of $Ca(OH)_2$+ saline solution, Calen ($Ca(OH)_2$/P) polyethylene glycol base, Calen camphorated paramonochlorophenol (CMCP) ($Ca(OH)_2$/CMPC), and $Ca(OH)_2$+ CHX pastes. The pH of the pastes was determined with a calibrated pH meter placed in direct contact with each paste. The root canals of acrylic teeth (n = 10) were filled with the previously mentioned intracanal dressings and immersed in ultrapure water to measure hydroxyl (pH meter) and calcium ion release (atomic absorption spectrophotometer) at time intervals of 3, 7, 15, and 30 days. To assess solubility, the root canals of acrylic teeth (N = 10) were filled with the previously mentioned pastes and scanned by micro-computed tomographic imaging before (initial) and after 7, 15, and 30 days of immersion in ultrapure water. The solubility of each specimen was the difference between the initial and final volume scanning. For antimicrobial analysis, monospecies and dual-species biofilms were induced on dentin blocks. Afterward, they were treated with the pastes for 7 days. Live/dead dye and a confocal microscope were used to measure the percentage of living cells. $Ca(OH)_2$/P and $Ca(OH)_2$/CMCP showed a higher percentage of Ca2+

ion release. $Ca(OH)_2$/CHX presented the greatest antimicrobial action. $Ca(OH)_2$/P and $Ca(OH)_2$/CMPC showed higher solubility values in the period analyzed.

Udaya Kumar et al[68] **in 2016** evaluated the Antibacterial efficacy of 2% CHX, N-acetyl cysteine (NAC) and assessed their synergistic or antagonist action as intracanal medicament. The diameters of zones of bacterial inhibition were measured and recorded for each solution. Sixteen freshly extracted teeth were vertically sectioned into two halves resulting in a total of 32 samples. The samples were inoculated with bacterial suspension and incubated at 37°C for 2 weeks for biofilm formation. The samples were then divided into four groups with 8 samples in each group. The samples were placed in culture wells containing the test solutions, i.e. 2% CHX, NAC, a combination of 2%CHX and NAC in 1:1 ratio, and a control group saline. The biofilm formed on the root canal surface was removed and checked for the formation of E.faecalis colonies. Agar diffusion test showed 2% CHX and NAC almost equal zones of inhibition.

Dhariwal et al[69] **2016** evaluated the antibacterial effectiveness of Sodium hypochlorite, Ethanolic extracts of Curcuma longa (turmeric) and Camellia sinensis (green tea) as irrigating solutions against the anaerobic bacteria isolated from the root canals of infected primary teeth**.** Root canal samples were taken with the help of sterile paper points transferred to tubes containing thioglycolate

transport medium. The bacteria was then isolated using standard microbiological protocols and were subjected to antibiotic sensitivity testing using the three test irrigants. The most commonly isolated bacteria included Porphyromonas sp, Bacteroides fragilis, Peptostreptococcus, and Staphylococcus aureus. Sodium hypochlorite and C. longa(turmeric) showed good antibacterial effect and were effective against most of the isolated bacteria. There was statistically significant difference in the antibacterial effect among the three tested groups and the least effective was C. sinensis (green tea).

Mitali et al[70] **2016** comparatively evaluated the antimicrobial efficacy of Curcuma longa (Tumeric), Azadiracta indica (Neem) and 3% sodium hypochlorite against E. faecalis as an endodontic irrigants. Agar plates were prepared using Brain Heart Infusion (BHI) agar and Cultures of E. faecalis grown at 37°C. The irrigants were divided into Group 1: Turmeric in Sterile Distilled Water (T+D), Group 2: Neem in Sterile Distilled Water (N+D), Group 3: Neem in Absolute Ethanol (N+E) Group 4: 3% NaOCl (control) Group 5: Absolute Ethanol (control) (E). Plates were inoculated for 24h at 37°C and microbial zones of inhibition were recorded. Wells of 6mm diameter were punched on the agar surface, to which 20 µl of each sample was added. The experiment was carried out simultaneously on 5 agar plates and the mean zones of inhibition were calculated. Sodium hypochlorite and Ethanolic extract of neem

showed maximum antibacterial activity against E. faecalis compared to aqueous turmeric and neem extract.

Bhagwat et al.[71] 2017 investigated antimicrobial potential of a Curcumin-containing product Dennkur, 3% Sodium Hypochlorite. 2% Chlorhexidine and Calcium Hydroxide against endodontic pathogens E.faecalis and C.albicans by Agar cup method. Sterile nutrient agar for Enterococcus Faecalis and Sterile Sabourauds Agar For Candida Albicans were taken. The agar medium was mixed with 1ml of bacterial strain and spread on petriplates to solidify at room temperature. 5 wells of 8mm diameter were punched and 0.1 ml of undiluted medicaments was placed inside. Zones of inhibition were recorded with the help of vernier caliper. DennkurTM showed zero zone of inhibition against E faecalis & C.Albicans as compared with the gold standard irrigants and medicament, which showed zones of inhibition ranging from 8-16mm. Thus commercial product DennkurTM in its present formulation appears to be ineffective in showing the similar antibacterial activity.

Nagamaheshwari et al[72] 2017 did a study to compare and evaluate antimicrobial efficacy of three medicament combination Calcium hydroxide (CH) powder and 2 percent Chlorhexidine (CHX) liquid (Group 1); CH, CHX and Curcuma long a[Turmeric powder (TP)] (Group 2); and CH, CHX and Retinoic acid [vitamin A (VA)] (Group 3) by Agar diffusion test. It was found that group 3 had

better results compared to other groups, thus Vitamin A can be used in combination with CA and CHX as medicament.

Jahanezade et al[73] **2017** developed a novel bio Nano composite of Carboxymethyl Starch (CMS)-Chitosan (CS) -Montmorillonite (MMT) for curcumin delivery. To improve Curcumin entrapment into Cs-CMS-MMT, different ratios of Chitosan (Cs), Carboxymethyl Starch (CMS) and MMT were used. The optimal formulation had the average particles size of 35.9 nm with Curcumin entrapment efficiency of 91%. Finally, the antibacterial activity of bio Nano composite against Streptococcus mutans was assessed. Curcumin-loaded bio Nano composite hindered the formation of biofilm on dental models very effectively.

Naseri et al[74] **2019** evaluated the micro hardness and superficial chemical structure of radicular dentin of Calcium hydroxide and Nano calcium hydroxide. Dentin micro hardness was assessed by the Vickers test, and the phosphate/amide ratio was evaluated by the Fourier-transform infrared spectrometry test. The use of CH as an intracanal medicament for 4 weeks reduced dentin micro hardness, whereas NCH did not result in any change in the micro hardness value. However, a change in the superficial chemical structure was observed earlier after 1 week in both the CH and NCH groups.

Ozurck et al[75] **2019** investigated the antimicrobial effects of six different intracanal medicaments on Enterococcus faecalis. Medicaments used were Calcium hydroxide $Ca(OH)_2$ with saline, $Ca(OH)_2$ with anesthetic solution, $Ca(OH)_2$ with propylene glycol, commercially available premixed $Ca(OH)_2$paste, Chlorhexidine gluconate gel, Triple antibiotic paste (metronidazole, ciprofloxacin, doxycycline) with propylene glycol and talk powder with saline as negative control group. The zones of inhibition of TAP was significantly larger compared to other groups and is preferred medicament against E.faecalis.

Ooi et al[76] **2019**compared the antimicrobial activity of pediocin with Chlorhexidine (CHX) and Calcium hydroxide $Ca(OH)_2$ against Enterococcus faecalis and Staphylococcus epidermidis biofilms. Bacteriocins are ribosomaly encoded proteinaceous molecules produced by bacteria of all genera to kill or inhibit the growth of other bacteria. Pediocins are cationic peptides that belong to class IIa bacteriocins. The CFU counts showed that pediocin showed the best antimicrobial activity compared to other groups. There was no growth in pediocin group and was highly effective.

Oda et al[77] **2019** evaluated the capacity of carbopol gel to maintain the intensity of a LED curing light (blueLED) along the length of prepared root canals in bovine teeth, and to assess the antimicrobial capacity of curcumin photoactivated by a LED curing light in the

presence of carbopol gel. In one experiment 8 bovine incisors were instrumented and LED-curing light was irradiated inside the root canals using an aluminium collimator (1.5mm in diameter) placed at the orifice. Initially irradiation was done in empty canals and later filled with carbopol gel. Simple photographs were taken and analyzed in software to verify light intensity in root length. Carbopol gel did not improve the intensity of LED light transmission along the root canal. In second experiment 20 blocks were prepared and subjected to standard PDT (methylene blue + diode Laser); Curcumin; LED curing light; and Curcumin + LED curing light. Bacterial viability was very much reduced for Curcumin + LED and standard PDT.

MATERALS AND METHODS

MATERIALS USED:

- 120 mandibular premolar teeth with single roots
- Calcium hydroxide paste
- 2% Chlorhexidine gel
- Curcumin powder (Sigma chemicals)
- Carbapol P934
- Deionized water
- Pluronic F127
- Ethanol 100mg/ml
- Polyethelene glycol PEG 400
- E.faecalis (ATCC)
- TSBG –Tryptic soy broth with 0.5% glucose
- DMSO – Dimethyl sulfoxide
- Mueller hinton broth
- 3% Sodium hypochlorite
- 17% Ethylene diamine teracetic acid
- Brain Heart Infusion broth

INSTRUMENT AND EQUIPMENT USED:

1. Ultrasonic scaler (woodpecker, China)
2. Diamond disc (Diamond Disc Pvt. Ltd, New Delhi, India)
3. Micromotor hand piece (NSK Corporation, Japan)

4. #15-25 K files (Mani inc, India)
5. Gates glidden drills # 2,3 and 4
6. Protaper gold rotary files (Dentsply Maillefer, Ballaigues Switzerland)
7. Digital Autoclave (Inlab equipment, Chennai, India)
8. Laminar air flow (Air Sys Technology, Chennai, India)
9. Magnetic stirrer
10. Amber bottles
11. Whatmann paper disc.
12. Eppendorf tubes (lab Tech, India)
13. Incubator
14. Culture plates
15. FTIR instrument (Shimadzu ,nfinity –Japan)
16. Hemocytometer
17. Ball miller (Retsch PM100)
18. Endo motor (X-Smartplus, Dentsply Maillefer, Japan)
19. Size 2 Lentulospiral (Mani inc, India)

Methodology of Nano Curcumin gel preparation

Curcumin nanoparticles were prepared by ball milling method (Retsch PM100). The particle size obtained was roughly around 30-50nm, which was determined by dynamic light scaterring. FTIR results showed that standard Curcumin and Nano Curcumin have similar functional groups, thus confirming that particle is converted

to nano particle. For gel preparation using a magnetic stirrer, one percent w/v Carbopol P934 was primarily dissolved in deionized water. Once dissolved the preparation was placed in an ice bath. With continuous mixing, Pluronic F127 (30% w/v) was slowly added. The mixture was kept in refrigerator for 24 hours at 4^{o}C for ensuring the complete moistening and removal of air bubbles trapped in it. To the already made polymer solution, Nano Curcumin powder 1% w/w, and 2% w/w dissolved in a suitable quantity of PEG 400 (140 mg/ml) was added by stirring in ice bath, after which 100 mg/ml ethanol (100 mg/ml) was added. Samples were transferred to amber bottles and kept inside the refrigerator. Nano Curcumin got dissolved in ethanol for the gel formulation and was kept on the stirrer whole night for evaporation of ethanol before storing in a refrigerator. The gel pH and viscosity was measured.[78]

Bacterial strain

The microbial strain Enterococcus faecalis were obtained from ATCC. The bacteria were cultivated in Tryptic soy broth supplemented with 0.5% glucose (TSBG) at 37°C with 5% CO_2 for 16–18 hours.

Determination of MIC

Nano Curcumin gels MIC was experimented via microbroth dilution procedure. A nano curcumin gel stock solution was made by taking the compound (2mg) in distilled water (1mL). To obtain a

concentration in between 0.05-3.0%, the stock solution was consecutively diluted in dimethyl sulfoxide DMSO (1mL). Similarly saline was used as the control. The culture were then incubated and subsequently, serially diluted to reach the density of 2 × 104 cells per ml. Cell counting was done using hemocytometer. Two milliliters of Mueller Hinton broth(MHB) was dispensed in tubes, and 100 µL of cell culture was inoculated in it. Then, different concentration of Curcumin gel (0.05-3.0%) was added to each tube. Growth control was run in parallel with every experiment. All the experimental tubes were incubated in anaerobic jars for 48 h. After completion of incubation period, the optical density was measured at 600 nm. Each experiment was carried out in a triplicate set. The lowest concentration prior to color change was considered as the Minimum Inhibitory Concentration (MIC).

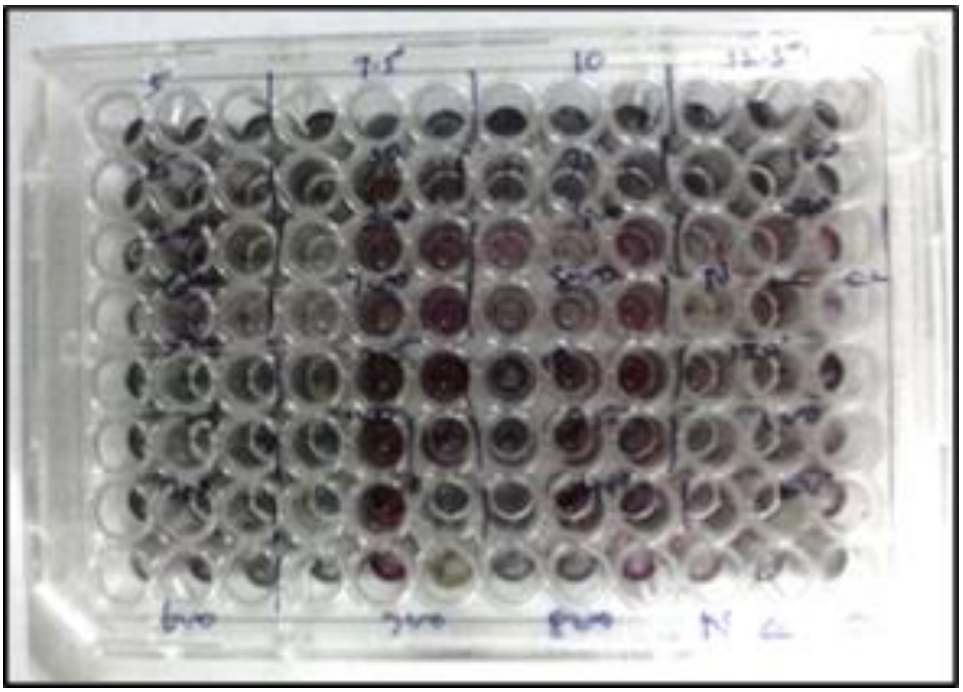

Fig 1: Determination of MIC by tube dilution method

Determination of Minimum bactericidal concentration:

To examine the antibacterial effects in contrast to the strain on Muller-Hinton agar, well- diffusion method was adapted. From the suspension, diluted inoculum (108 CFU/ml, 100µL) was taken and was spread on the plate surface and they were kept for solidifying. Under aseptic conditions, the Whatman paper disc (6mm in dm) was soaked in curcumin gel, (0.5-3%) and DMSO (control). For bacterium, the plates were kept at 37°C for 24 hours. By observing the diameter ZOI contrast to the test microbes, the antimicrobial effect was assessed. Every test was repeated thrice in similar methodology.

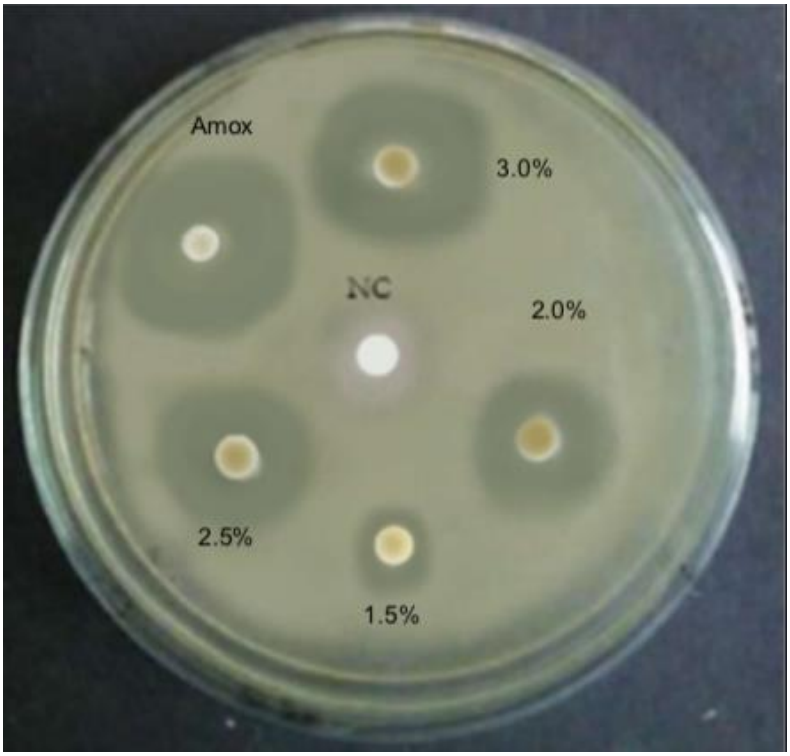

Fig 2: Determination of MBC by disc diffusion method NC- Negative control; NCu G-Nano Curcumin gel (1.5; 2.0; 2.5; 3.0%); Amox-Amoxiillin

Selection of samples

Inclusion criteria:

Intact Mandibular Premolar extracted for therapeutic purpose

Free of caries

Absence of severe root curvature

Straight canals with type I classification

Absence of fracture

Exclusion criteria:

Calcified canals

External and internal root resorption

Fractured teeth

Preparation of Root samples:

120 freshly extracted human mandibular single rooted premolar teeth with type I classification that was confirmed by taking radiograph were collected from patients undergoing therapeutic extraction in Department of Oral and Maxillofacial Surgery, Chettinad Dental College. The collected samples were caries free and devoid of any anomalies. Samples were washed in running water and soft tissue remnants, debris or calculus was removed. They were placed in hydrogen peroxide (H_2O_2) solution for 1 hour after which it was transferred to normal saline until use.

The teeth were decoronated using diamond disc below Cemento enamel junction to standardize working length to 14mm. 15K file was used to negotiate the teeth upto working length, Biomechanical preparation was done using NiTi rotary files upto F3(Protaper gold, Denstply)following normal irrigation protocols (3% NaOCl & 17% EDTA). Final irrigation of the samples were done with 5ml-distilled water and placed in ultrasonic bath for 5min to remove traces of any chemicals used. The samples were placed in distilled water for 5minutes after which they were autoclaved for 15min at 121^{O}C. Pure culture (4h) of E. faecalis were suspended in of Brain Heart Infusion broth (BHI 5 ml) and kept at 37°C for 24 hours in a incubator.

Samples incubated with E.faecalis

The sterilized root samples were then aseptically shifted onto separate micro centrifuge tubes having Brain Heart Infusion broth (BHI 1 ml). The 24h isolated colonies of E. faecalis culture (ATCC 29212) developed on Tryptone soya agar has been suspended in 5ml of Tryptone soy broth. It was then kept at 37°C for 4h. Bacterial culture suspensions (50 µl) was added onto every tube and kept for 48 hours at 37°C. These Samples were then shifted to a fresh broth having bacterial culture, every alternate day. Between this, randomly 12 test samples were taken for analyzing the microbial colony count. After incubation both exterior and interior surface of the samples were irrigated with 5ml Sterile Saline under aseptic

condition. Nail varnish layers were applied on the exterior specimen surface for preventing the medicament contact to the outer surface.

Antimicrobial Assessment

The remaining 108 samples were randomly divided into four groups of 27 each (Group I- IV). In Group I no medicament was placed and was only rinsed with 5ml sterile saline. In Group II the canals were dried with sterile paper points and Calcium Hydroxide paste was placed with the help of size 2 Lentulospiral. In Group III -2% CHX gel and Group -IV 3% Nano Curcumin gel were placed respectively in similar methodology.

Using paraffin wax, upper and lower part of the root samples were sealed and it was kept at 37°C in aerobic atmosphere. Randomly 9 samples from each group were taken at intervals of day 1, 3 and 7.The medicaments were thoroughly removed with help of 5ml saline and ultrasonic. With the help of Gates Glidden drill No. 3 &4 dentin debris were collected at two depths (200 &400 μm) and placed in BHI broth (1 ml) for 24h at 37°C. After incubation CFU w ere counted using digital colony counter.

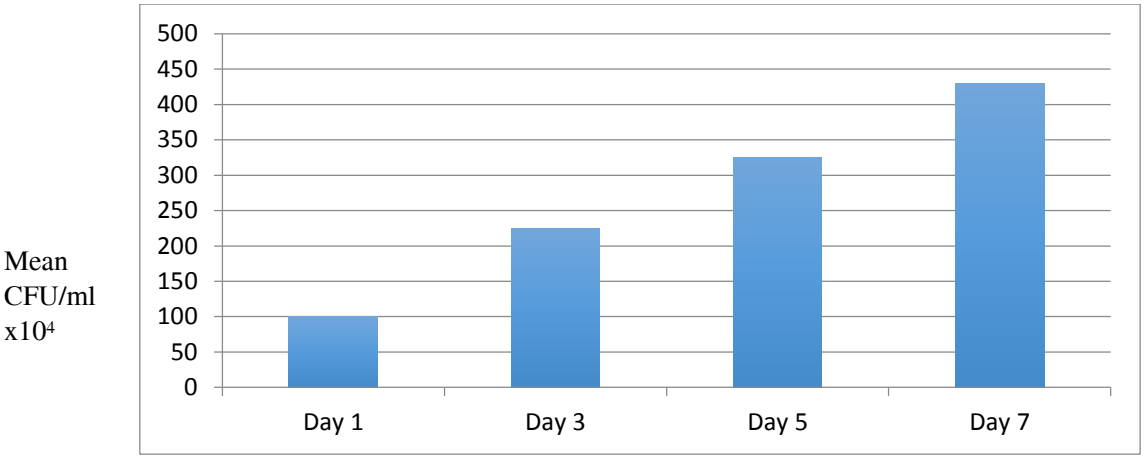

CHART 1: Microbial contamination of E. faecalis in test samples. At the end of day 7 the samples showed maximum contamination.

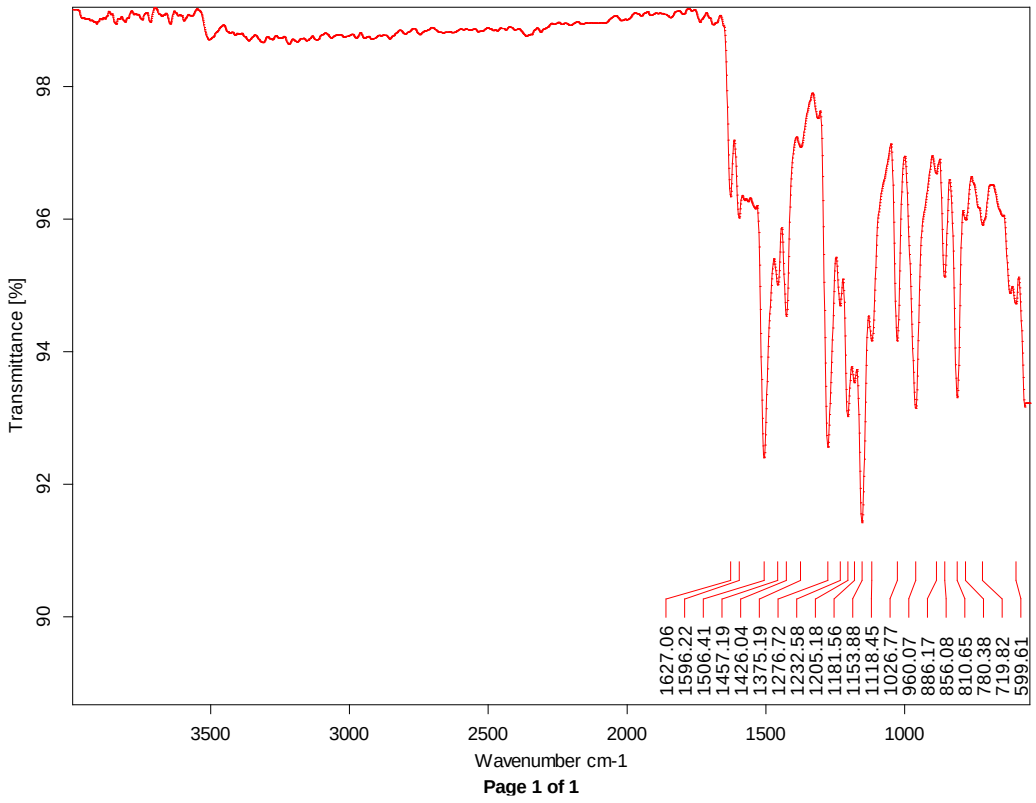

Fig 3: FTIR analysis of standard Curcumin

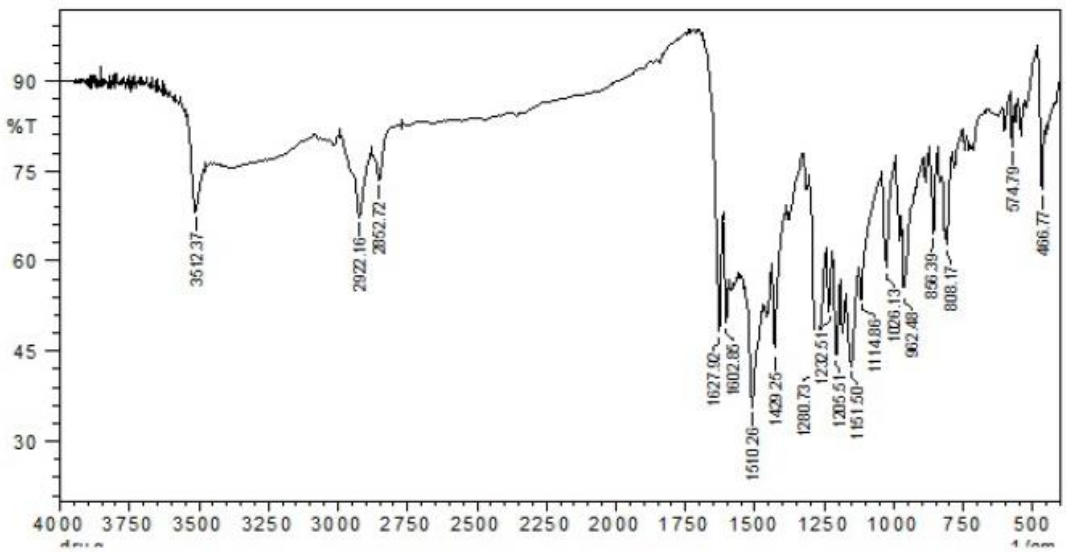

Fig 4: FTIR analysis of Nano Curcumin.

Fig 5: Decoronation using diamond disc

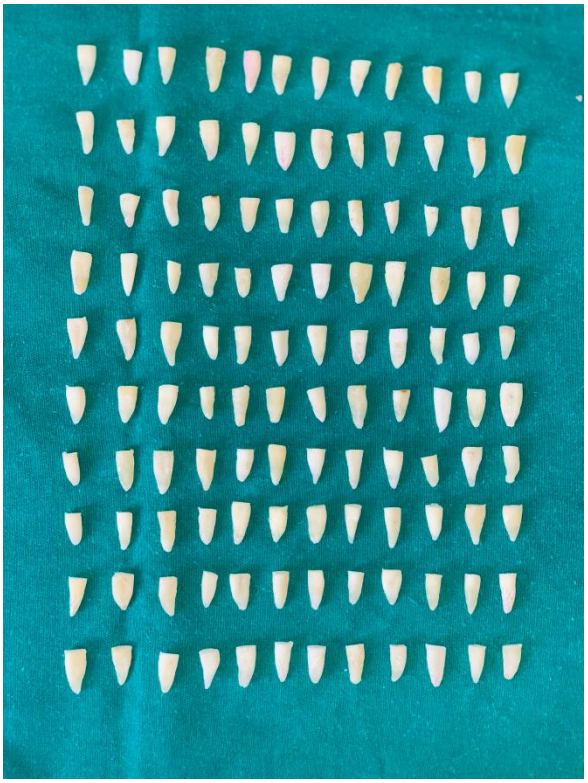

Fig 6: 120 Decoronated extracted mandibular Premolar teeth.

Fig 7: Ultrasonic bath agitation after biomechanical preparation.

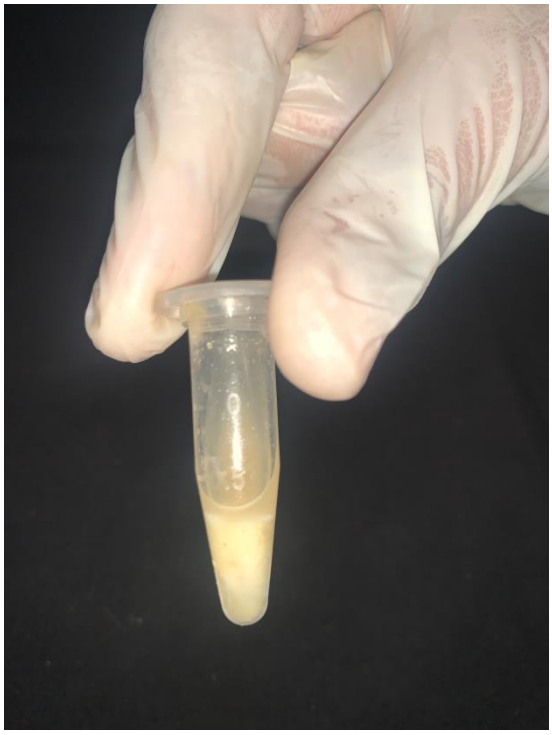

Fig 8: Root samples soaked in E.faecalis broth.

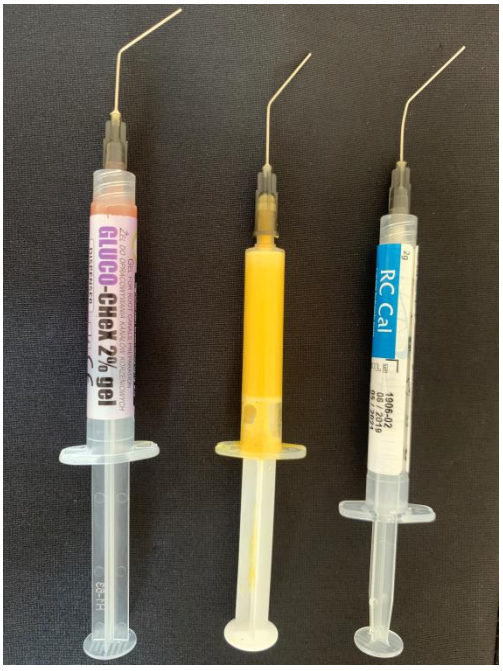

Fig 9: Intracanal medicaments 2% CHX (gluco chex gel), 3% Nano curcumin gel and Calcium hydroxide paste.

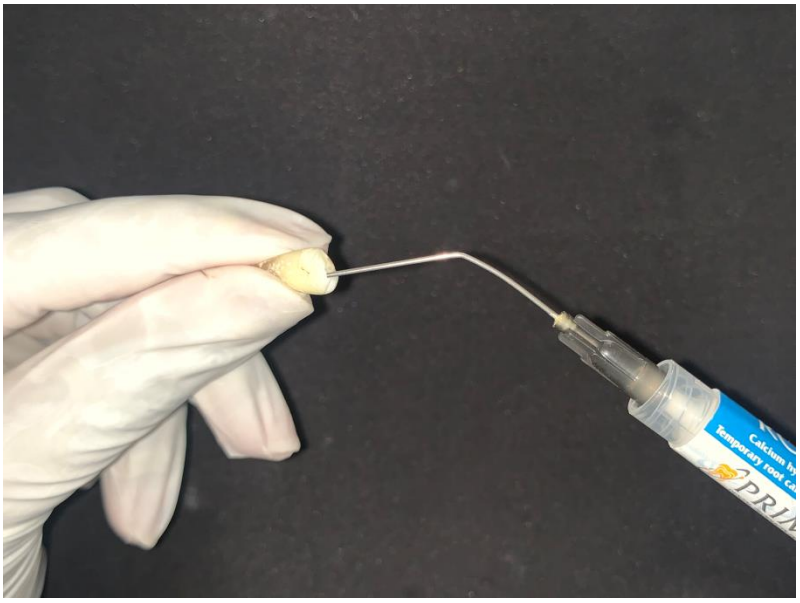

Fig 10: Placement of medicament

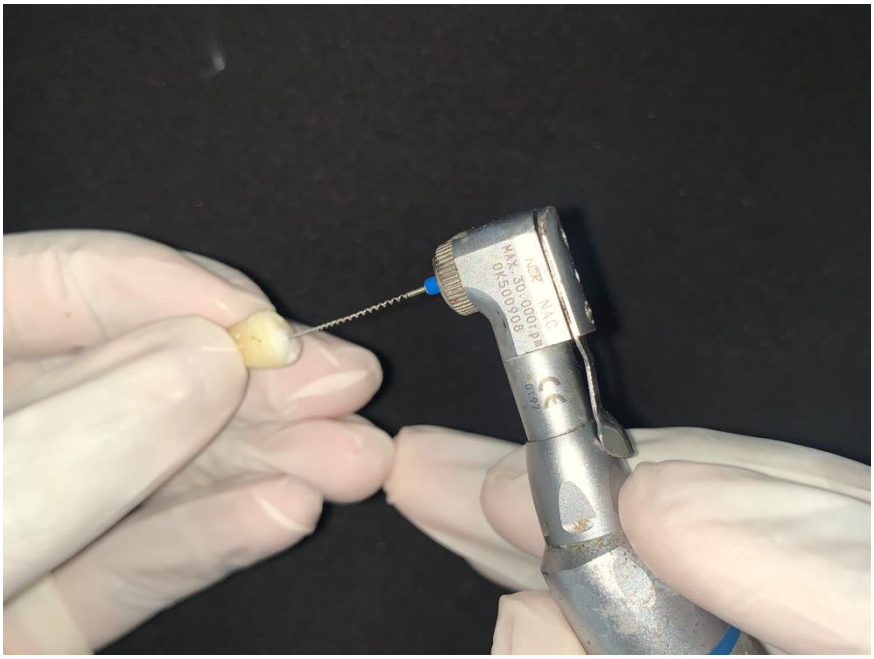

Fig 11: Final placement done using lentulo spiral

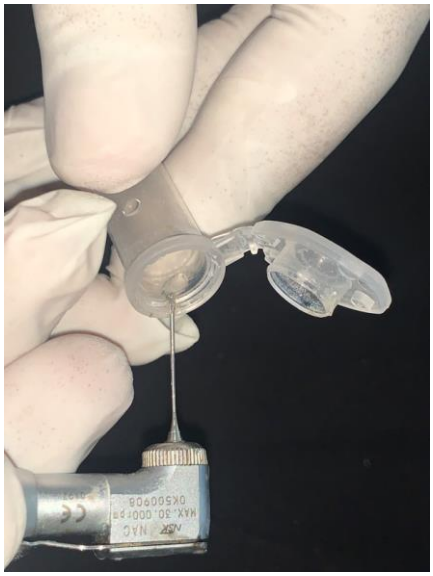

Fig 12: Dentin debris collection using Gates Glidden drills.

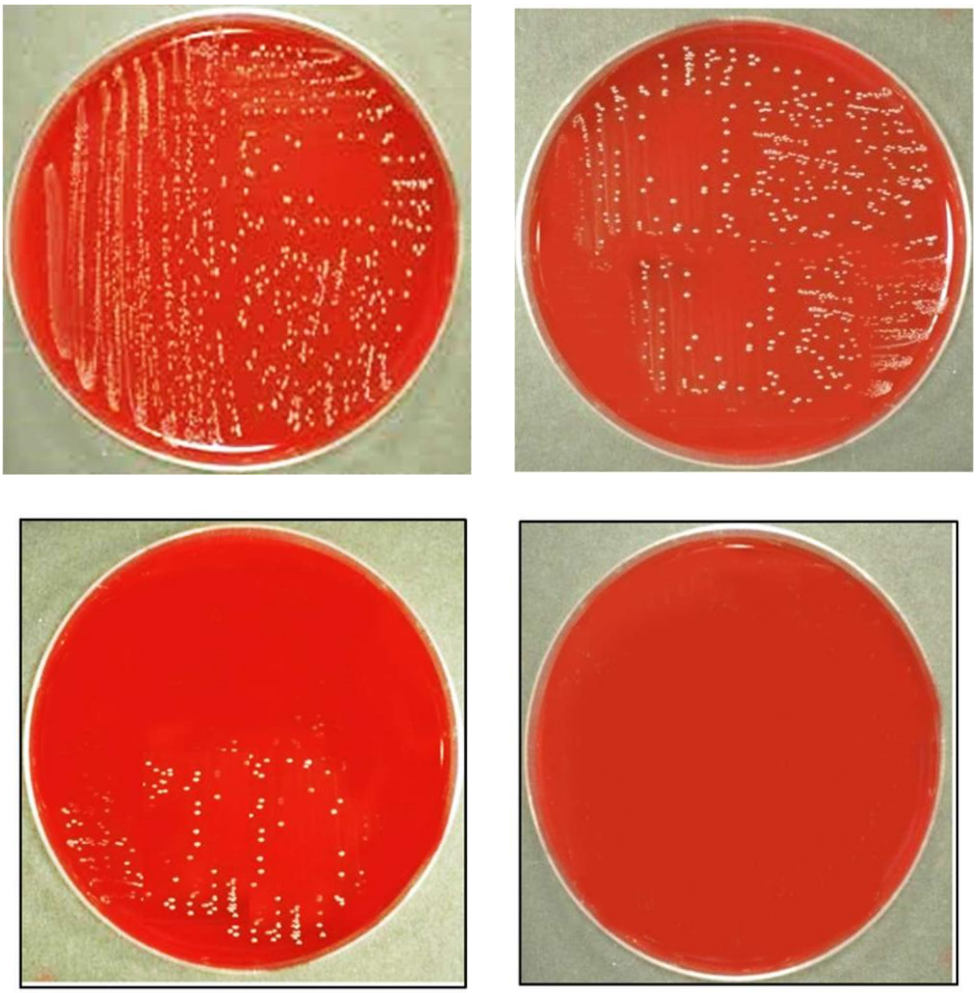

Fig 13: Enterococcus faecalis microbial colony in blood agar plates; A- Negative control; B- $Ca(OH)_2$: C- N Cur gel: D-CHX

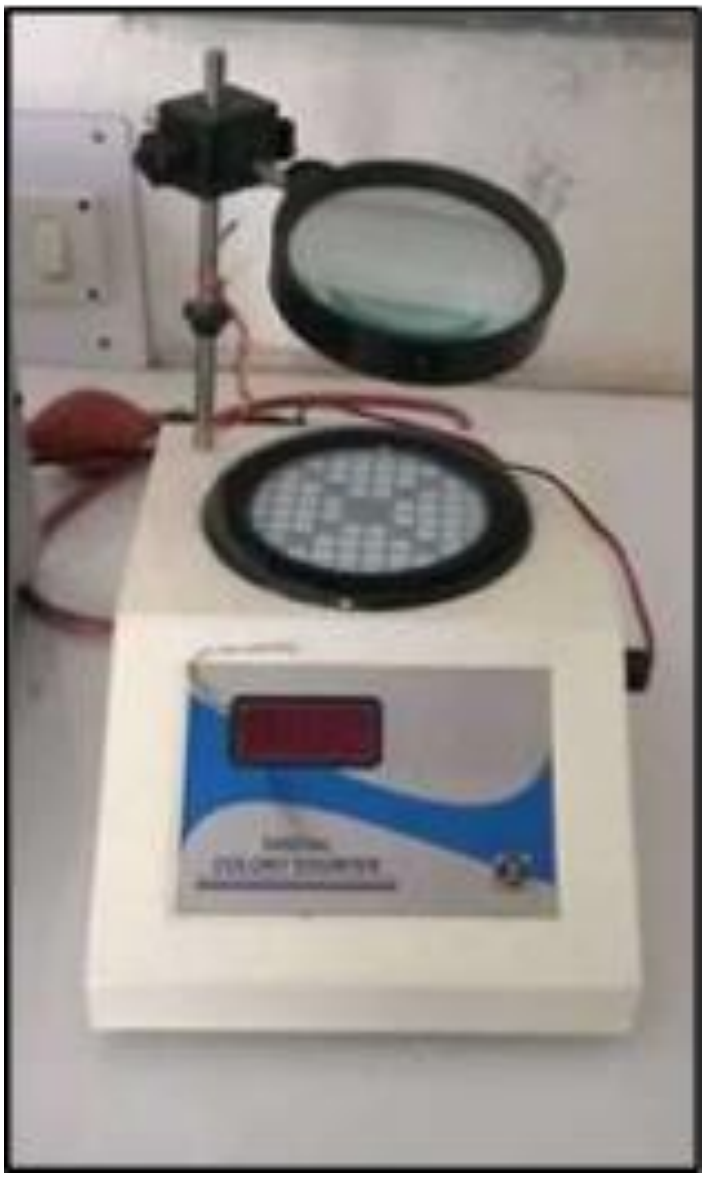

Fig 14: Digital colony counter

STATISTICAL ANALYSIS

For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukeys post hoc analysis. Statistical significance was accepted at a level of $P<0.05$. Data were analyzed using SPSS (version 22.0)

RESULTS

Table 1 shows percentage of E.faecalis present following treatment with various concentration of Nano Curcumin. It was found that 1.5% cucumin was sufficient in bringing about inhibition of E.faecalis, which was significant compared to control group. Each value is expressed as Mean ± SD(n=3). P value was compared with saline control. F value -8.706; MIC – 1.58 %

Chart 2 shows percentage of Nano curcumin required to inhibit growth of E.faecalis. 1.5% of Nano curcumin gel reduced nearly 50% of bacteria and 3% showed nearly 90% inhibition. Thus for this study 3% Nano curcumin gel was used.

Table 2 shows diameter of inhibitory zone caused by various medicaments. Its seen that 2-3% Nano curcumin gel showed wide diameter of inhibition, NI means no inhibition zone. Each value is expressed as mean ± SD (n = 3). *p value statistically significant as compared with negative control. F value -8.76.Thus it was concluded that **MBC of nano curcumin is 2.5%**

Chart 3 shows that Nano curcumin 2% and 3% showed wide diameter of inhibition compared to 1.5% gel.

Table 3 shows Microbial colony count at various depths at different time intervals with various medicaments. Results are expressed as

Mean ± SD [b]p<0.01; [c]p<0.001 statistically significant as compared with negative control. F value for Day 1 – 6.72; Day 3- 7.31; Day 7- 9.26.

Table 4 shows intergroup comparison at depth of 200µm and it is seen that there is a significant difference in microbial colony count between the groups. On Day 1, significant difference is found between each group versus all other groups. On day 3, significant difference is found between each group versus all groups except between CHX and Nano Curcumin (Tukeys post hoc test). On Day 7, significant difference is found between each group versus all other groups.

Chart 4 shows microbial colony count at 200 µm after placing the medicaments, it shows that all medicaments had significant efficacy compared to saline group.

Table 6 shows intergroup comparison at depth of 400µmand is seen that on Day 1, significant difference is found between each group versus all other groups. On day 3, significant difference is found between each group versus all groups except between CHX and NanoCurcumin. On Day 7, significant difference is found between each group versus all other groups.

The data of Nano Curcumin is much spread out around Mean/ Central tendency and confidence interval includes a negative sign which contains true value of Nano curcumin, this means that data shows much variability compared to others. The confidence interval cannot be considered as statistically significant and there is no significant difference between Curcumin and CHX on day 3.

Chart 5 shows microbial colony count at 400 µm after placing the medicaments, it shows that all medicaments had significant efficacy compared to saline group

To summarize the results, the overall percentage inhibition at 2 depths and different time intervals was 100% with 2% chlorhexidine gel, 60% for Nano curcumin gel and 40% for calcium hydroxide.

Plant extract	Conc (%)	E. faecalis	% 0f Inhibition	P value
Saline	-	0.412 ± 0.025	-	
N Cu gel	0.05	0.307 ± 0.03	74.51	0.005
	1.0	0.238 ± 0.00	57.76	0.0099
	2.0	0.104 ± 0.11	25.24	0.001
	3.0	0.056 ± 0.04	13.59	0.001
Amoxicillin (µg)	10	0.035 ± 0.06	8.49	0.001

Table 1: Minimum inhibitory Concentrations of Nano Curcumin gel and Amoxicillin against E.faecalis.

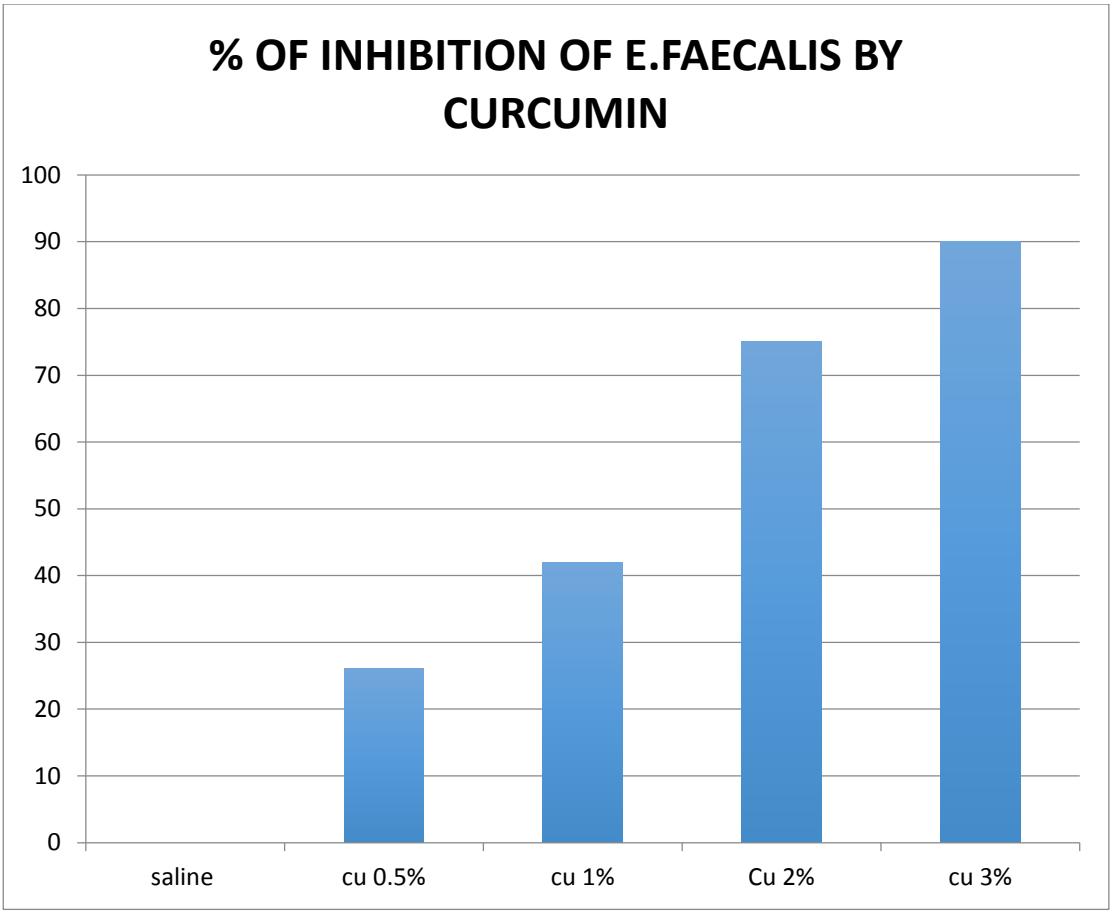

CHART 2: Percentage of E.faecalis inhibited by various concentrations of Nano Curcumin

Samples	Conc	E. faecalis Zone of Inhibition (mm)	P value
NC		NI	
Cur gel (%)	1.5	8.5 ± 0.26*	0.999
	2.0	10.4 ± 0.92*	0.0010
	2.5	12.1 ± 0.85*	0.0010
	3.0	14.5 ± 0.71*	0.0010
Amox (µg)	10	16.4 ± 1.2*	0.0010

TABLE 2: Diameter of inhibition of different Nano curcumin concentrations. MBC-2.5%

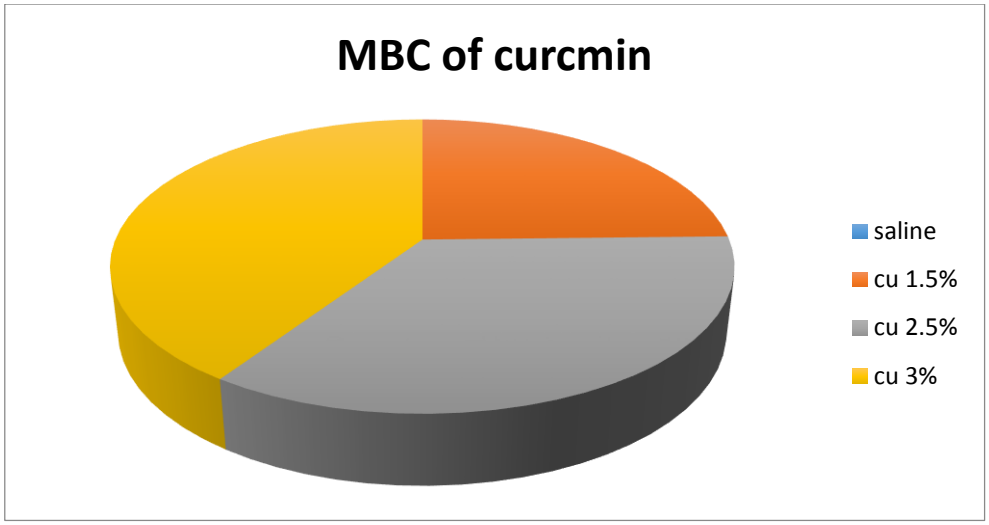

CHART 3: Zone of bacterial depletion of different Nanocurcumin concentration.

Microbial colony count (10^5)						
Groups	**Day 1**		**Day 3**		**Day 7**	
	200 µm	**400µm**	**200 µm**	**400µm**	**200 µm**	**400µm**
Saline	2.87± 0.18	2.94± 0.12	2.45± 0.21	2.71± 0.19	2.12± 0.12	2.58± 0.13
Ca (OH) 2	1.87± 0.07^b	1.96± 0.08^b	1.42± 0.13^b	1.78± 0.10^b	1.37± 0.10^b	1.43± 0.08^b
CHX 2%	0.0± 0.0^c	0.0± 0.0^c	0.0 ± 0.0^c	0.0± 0.0^c	0.0 ± 0.0^c	0.0± 0.0^c
Nano Curcumin gel (3%)	0.56± 0.06^c	0.89± 0.05^c	0.48± 0.02^c	0.53± 0.04^c	0.41± 0.03^c	0.43± 0.04^c

TABLE 3: Microbial colony counts of various medicaments at different time interval and depth

Day	Group	Mean	SD	95% CI for Mean		F	P value
				Lower	Upper		
Day 1	Saline	2.87	.11	2.59	3.16	132.6	<0.001
	Ca OH	1.87	.32	1.06	2.67		
	CHX	.000	.00	.000	.000		
	Curcumin	.560	.18	.104	1.01		
Day 3	Saline	2.45	.31	1.67	3.22	32.9	<0.001
	Ca OH	1.42	.47	.238	2.60		
	CHX	.000*	.00	.000	.000		
	Curcumin	.480*	.32	-.32	1.28		
Day 7	Saline	2.12	.17	1.68	2.55	177.1	<0.001
	Ca OH	1.37	.11	1.08	1.65		
	CHX	0.000	.00	.000	.000		
	Curcumin	.413	.13	.075	.751		
* = Not significant in pairwise comparison (Tukey's post hoc test)							

TABLE 4: Descriptive statistics and intergroup comparison by One-way ANOVA results for data at 200µm

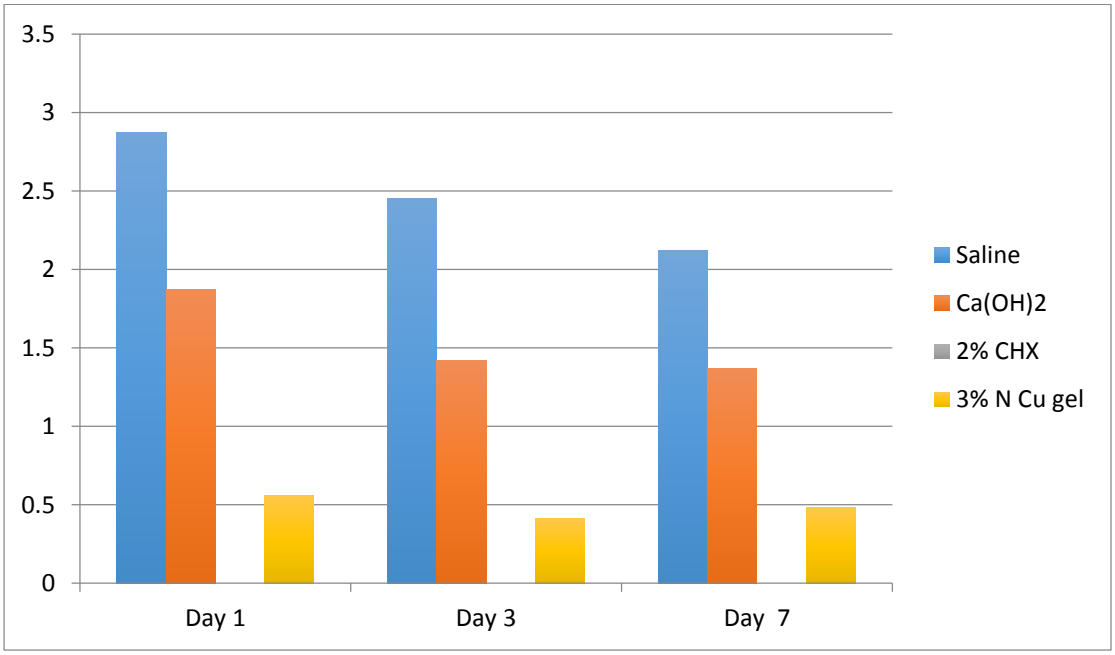

CHART 4: Microbial Count at 200 µm

Day	Group	Mean	SD	95% CI for Mean		F	P value
				Lower	Upper		
Day 1	Saline	2.94	.107	2.67	3.21	73.2	<0.001
	Ca OH	1.96	.350	1.08	2.83		
	CHX	.000	.000	.000	.000		
	Curcumin	.890	.365	-.019	1.79		
Day 3	Saline	2.71	.216	2.17	3.24	55.9	<0.001
	Ca OH	1.78	.445	.674	2.88		
	CHX	.000*	.000	.000	.000		
	Curcumin	.533*	.275	-.14	1.21		
Day 7	Saline	2.58	.087	2.36	2.79	385.1	<0.001
	Ca OH	1.43	.174	.996	1.86		
	CHX	.000	.000	.000	.000		
	Curcumin	.430	.055	.291	.568		
* = not significant in pairwise comparison (Tukey's post hoc test)							

Table 5. Descriptive statistics and intergroup comparison by One-way ANOVA results for data at 400µm

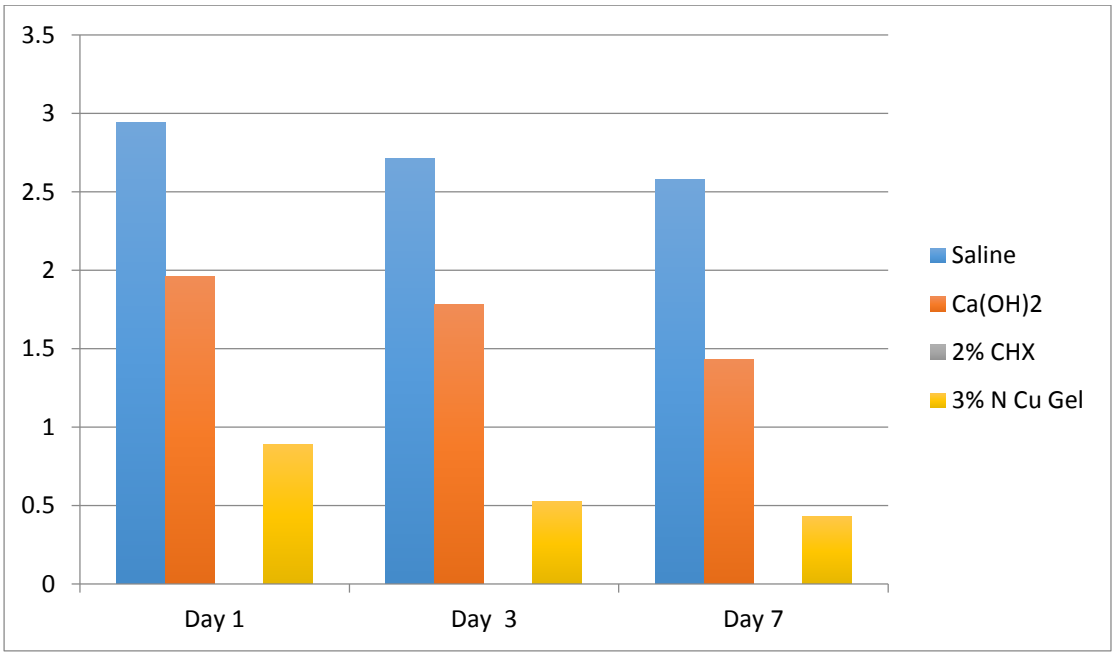

CHART 5: Microbial Count at 400 µm

DISCUSSION

Enterococcus faecalis is the principle organism in failed endodontic cases as it can survive high temperature, pH and can invade the dentinal tubules. Nearly 80% of the bacteria in chronic endodontic infection are E.faecalis[28]. Hence E.faecalis was used as test organism and was subjected to various medicaments. There are several reasons for bacterial survival after placing intracanal medicament. Firstly, some bacterial strains can be intrinsically resistant to medicament. Secondly, sometimes the medicament cannot reach the complex anatomy of root canal as a result of which bacteria can survive. Thirdly, tissue components and bacterial cell components will neutralize the medicament. Finally, placing the medicament for short duration leading to its inefficacy[49].

Biomechanical preparation alone cannot eliminate microorganisms from the root canal, as it is a complex system. Several intracanal medicaments have been used between appointments to eliminate microorganisms[79]. According to Byström et al[80] if the canal is left disinfected in case of multiple visit endodontics there is high chances that bacteria can multiply. This bacterial count can be maintained to a minimum if intracanal medicament is placed. The use of Intracanal medicaments in multiple visit endodontics is considered important as it controls the multiplication of remaining residual bacteria and also prevents re infection and inflammatory root resorption[79]. Most commonly used intracanal medicament is Calcium hydroxide and CHX. However limited antimicrobial efficacy is seen in calcium hydroxide against E.faecalis. This was confirmed in a recent systemic review and Meta analysis done by Sathorn et al[81]. The ineffectiveness of $Ca(OH)_2$ is mainly attributed to the bacteria, which can invade the dentinal tubules and survive high pH produced by the medicament[82]. Dentin also has some amount of buffering action on high pH, which further

compromises the action of calcium hydroxide[83]. The next most commonly used medicament was 2%chx gel. Portenier did a study and found that dentin matrix and type I collagen inhibited the action of CHX[84]. So to overcome the drawbacks of commercially available intracanal medicaments natural medicaments have been introduced. Curumin has potent antibacterial efficacy and has been used as intracanal medicament[18], however standard Curcumin had several disadvantage, to overcome that in the present study Nano preparation of curcumin was used.

The present study was done to evaluate and compare the antimicrobial efficacy of Curcumin nano particle hydrogel, Calcium hydroxide paste and 2% Chlorhexidine gel in dentin blocks infected with E.faecalis.

2% Chlorhexidine gel showed potent antimicrobial action at 3,5 & 7 days. This is mainly because it is hydrophobic and lipophilic molecule that will interfere with phospholipids and lipopolysaccharides on cell membrane of bacteria[85]. The positively charged CHX molecule will alter the osmotic balance by interfering with negatively charged phosphate group in bacterial cell wall leading to cell death[15]. One more reason why CHX exerts antimicrobial efficacy even at the end of 7th day is that it can absorb into the dentin surface and prevent microbial colonization[47].

In vitro study by Gomes et al[86] tested various concentrations of CHX gel (0.2%, 1%and 2%) and found that 2% gel formulation of CHX killed E.faecalis in 1min. Basrani et al[87] evaluated the antimicrobial efficacy of 0.2% and 2 % CHX gel and combination of calcium hydroxide with CHX against E.faecalis. It was found that 2%CHX gel was superior compared to 0.2% CHX. This is mainly due to the fact that 2% CHX had better substantivity compared to 0.2%CHX.

CHX does not have any tissue dissolving properties. In 2004 Naenni et al[88] did a study to find out the tissue dissolving capacity of 10%CHX, NaOCl, 10% per acetic acid and 30% hydrogen peroxide, It was found that none of the agents except NaOCl had tissue dissolving property.

Porteneir et al[84] tested the inhibitory effect of dentin matrix, type I collagen and iodine potassium iodide on 0.2%CHX, it was concluded that dentin components (HA and collagen) and inflammatory exudates reduced or inhibited the antibacterial activity of CHX.

Calcium hydroxide has a pH of 12.5 as a result of which it will kill the bacteria when placed in direct contact[89]. Antibacterial efficacy is mainly due to release of hydroxyl ions which damages bacterial cytoplasm, DNA and causes protein denaturation[90].

Calcium hydroxide is less soluble in water and can remain in canal for a long period of time, thus delaying bacterial progression towards the apical foramen[91]. It also acts as a physical barrier, which kills the bacteria by limiting the substrate for growth. It also keeps the wound area and periapical tissues free of bacteria thereby causing normal healing to occur[92].

The antimicrobial spectrum of calcium hydroxide is very limited and it does not affect all species of microbial flora in persistent endodontic infection. It is less effective against E.faecalis and it also has certain biocompatibility issues when it is pushed beyond root apex leading to toxicity[93]. Manzur et al[94] and Oncag et al[96]compared antibacterial efficacy of calcium hydroxide and CHX and found that CHX was more effective.

Turmeric is mainly composed of curcuminiods, which is nothing but curcumin diferuloyl methane, demethoxycurcumin and bisdemethoxycurcumin, which are polyphenols with strong antioxidant property[19]. Curcumin decreases the activity of filamenting temperature-sensitive mutant Z (FtsZ) protofilament and enhances GTPase activity. This change in balance is lethal to the bacteria[95]

Standard curcumin formulation was less solubile in water so when it is converted in nano form the solubility increased. Standard curcumin has a particle size ranging from 500-800nm, which is much higher compared to nano curcumin, which has particle size ranging from 30-50nm. Enhanced antimicrobial efficacy and solubility of Nano curcumin could be due to increased incorporation of nano particles in small volume of vehicle[23,99].

In 2011 Haukyik et al[97] evaluated the phototoxic effect of increasing concentration of Curcumin with Polyethyleneglycol against E.faecalis and found that all samples of Curcumin exhibited phototoxic effect.

Ribeiro et al[98] evaluated antimicrobial efficacy of curcumin combined with photodynamic therapy. MRSA Results concluded that combination Curcumin showed 100% elimination of bacteria.

Neelakantan et al[100] compared efficacy of Curcumin and sodium hypochlorite against E.faecalis and C.albicans. He concluded that the antimicrobial effect of Curcumin was similar to sodium hypochlorite and can be used as an alternative for sodium hypochlorite.

In present study, widely used CFU counting was followed. One main advantage of CFU is that only viable bacteria are counted while dead bacteria are excluded. Sometimes clumps of bacteria can

be counted as single colonies, which is one limitation of this technique[101].

On day 1,3 and 7 2%CHX had the best antibacterial efficacy compared to Nano Curcumin and $Ca(OH)_2$. Clorhexidine has low contact angle with dentin and it diffuses into the tubules at a faster rate compared to other medicaments[102]. The next effective medicament was Nano Curcumin gel, however the efficacy was less compared to CHX due to buffering ability of dentin. Curcumin is stable at PH of 6.5 but highly unstable at alkaline pH and forms crystalline structures at acidic pH, which can be a reason for decreased efficacy compared to CHX[104,105]. The least effective medicament was $Ca(OH)_2$. The ineffectiveness is mainly due to buffering action of dentin and dentin components, which reduces the pH of $Ca(OH)_2$ as a result of which bacteria can survive. Another reason is that $Ca(OH)_2$ has low diffusibility into dentinal tubules compared to other medicaments[103].

One limitation of Curcumin is that, it caused staining of the root canal walls. To prevent this, Nano Curcumin was used but some amount of staining did occur which was less compared to standard Curcumin. As ball-milling technique was implicated in present study, the particle size could be reduced to size of 40-50nm. According to Bhawana et al[23], Wet milling technique resulted in finer particles of size 10-20nm which significantly improved the solubility and did not cause any discoloration. In the present study 3% gel was used, may be higher concentration and prolonging the duration may have yielded better results.

SUMMARY

This In vitro study was done to compare and evaluate antibacterial efficacy of Curcumin nano particle hydrogel, 2% Chlorhexidine gel and Calcium Hydroxide paste as intracanal medicament against Enterococcus faecalis

Curcumin nano particles were prepared by ball milling technique after which it was prepared into a gel form using Polyethylene glycol. Minimum inhibitory concentration and Minimum bacterial concentration of the prepared gel was analysed. Thus based on the results 3% Curcumin nano particle hydrogel was tested in present study. 120 freshly extracted premolar teeth were decoronated and biomechanical preparation was done following normal irrigation protocol and divided into following groups Group I-Normal saline, Group II Calcium hydroxide paste $Ca(OH)_2$, Group III 2% Chlorhexidine gel (2%CHX) and Group IV 3% Nano curcumin gel (3% N Cu gel). The samples were inoculated with E.faecalis (ATCC) for 2 weeks after which the medicaments was placed inside the canal (n=27) with the help of size 2 lentulospiral. After treatment, dentin shaving was collected at end of day 1, 3 and 7 at varying depth of 200µm and 400µm in BHI broth. Colony forming units was counted with the help of digital colony counter.

Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukeys post hoc analysis. Statistical significance was accepted at a level of $p<0.05$. Data were analyzed using SPSS (version 22.0)

The results showed that Group 3 (2%CHX) was the most effective against E.faecalis on all days. Group 2 $Ca(OH)_2$ and Group 4 (3%N Cu gel) were statistically significant from the control Group 1. Mean ± SD, $^{b}p<0.01$; $^{c}p<0.001$.On Day 1, significant difference is

found between each group versus all other groups. On day 3, significant difference is found between each group versus all groups except between CHX and Nano Curcumin. On Day 7, significant difference is found between each group versus all the groups

Within the limitation the study it can be concluded that 2%CHX gel was most effective intracanal medicament. Nano Curcumin gel can be used as an Intracanal medicament as the efficacy is almost similar to commercially available intracanal medicaments.

CONCLUSION

Within the limitations of current study it can be concluded that 2%CHX was the most effective intracanal medicament against E.faecalis. Nano Curcumin can be considered as an alternative to commercially available Intracanal medicaments as it has comparable efficacy and it can be easily removed from the canal compared to widely used Calcium hydroxide. Use of herbal products is on the rise in field of dentistry and should be encouraged in future.

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ANNEXURE I

PARTICIPANT INFORMATION SHEET (ENGLISH)

NAME OF INVESTIGATOR: RAMEEZUDDIN

PHONE NO : 9677005828

TOPIC : COMPARISON AND EVALUATION OF ANTIMICROBIAL EFFICACY OF CURCUMIN NANOPARTICLE HYDROGEL, CALCIUM HYDROXIDE PASTE AND 2%CHLORHEXIDINE GEL AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS : AN IN VITRO STUDY .

The aim of the study is to use curcumin as intracanal medicament on extracted teeth . No risk will be involved as the teeth is extracted for orthodontic or periodontal problems . Your records will be maintained confidential and you have freedom to participate or withdraw from research at any point of time.

The details of the research has been explained to me in a language which I under stand . I hereby give permission for using my records, extracted teeth for professional research and education purpose only .

SIGNATURE OF PATIENT :-

ANNEXURE II

PARTICIPANT INFORMATION SHEET (TAMIL)

முதன்மை ஆய்வாளரின் பெயர்-ரமீஸுதீன்

கைப்பேசி என் - **9677005828**

தலைப்பு: மூன்று வகையாக நுண்ணுயிர் கொல்லியை பற்களின் வேர் பாதையில் செலுத்தி அதன் பலாபலன்களை ஆய்வு செய்வதே இந்த ஆராய்ச்சியின் குறிக்கோள் .

இந்த ஆராய்ச்சி புடுங்கப்பட்ட பற்களில் மேற்கொள்ளப்படுவதால், எவ்வித பாதிப்பும் ஏற்படாது என்றும் பல் சீரமைப்பு அல்லது ஈறுநோய் பாதிப்பினால் பிடுங்கப்பட்ட பற்களில் மட்டுமே இந்த ஆராய்ச்சி மேற்கொள்ளப்படும் என்று உறுதியளிக்கிறேன்.

தங்களது பதிவுகள் ரகசியமாக பாதுகாக்கப்படும் என்றும் தாங்கள் விருப்பமின்றி தங்களுக்கு பயன்படுத்தபடாது என்றும் உறுதியளிக்கிறேன்.

இந்த ஆராய்ச்சியின் குறிப்புகள் எனக்கு தெளிவாக விளக்கப்பட்டன, அவற்றை நான் முழுமையாக புரிந்து கொன்டேன்

இந்த ஆராய்ச்சிக்காக எனது பிடுங்கிய பற்களை உபயோகிக்கலாம் என முழுமனதுடன் சம்மதிக்கிறேன்.

நோயாளியின் கையொப்பம் -

ANNEXURE III

PARTICIPANT INFORMED CONSENT FORM (PICF)-ENGLISH

IHEC Proposal S.No: 384 Date:_____

Title of the project:

COMPARISON AND EVALUATION OF ANTIMICROBIAL EFFICACY OF CURCUMIN NANOPARTICLE HYDROGEL , CALCIUM HYDROXIDE PASTE AND 2%CHLORHEXIDINE GEL AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS : AN IN VITRO STUDY .

Name of the Principal Investigator: RAMEEZUDDIN.T Mobile No: 9677005828

The contents of the information sheet dated _____ that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from CARE. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

Date:

_____

Place:

(Signatures /Left Thumb Impression)

Name of the Participant:_____

Son/Daughter/Spouse/of_____

Complete Postal Address:

_____

_____

This is to certify that the above consent has been obtained in my presence.

Date:

_____ Place:

Signature of the principal Investigator

Witness – 1	Witness – 2
_____	_____
Signature	Signature

Note 1: Three copies should be made, for (a) Participant, (b) Researcher, (c) Institution 2: Submit the modified participants informend consent as per the study proposal 3: Investigators are advised to prepare the translation in simple understandable

Tamil on their own

ANNEXURE IV

PARTICIPANT INFORMED CONSENT FORM (PICF)-TAMIL

முறையான அனுமதி படிவம்

தேதி: _____

தலைப்பு: மூன்று வகையாக ங்ண்ணுயிர் கொல்லியை பற்களின் வேர் பாதையில் செலுத்தி அதன் பலாபலன்களை ஆய்வு செய்வதே இந்த ஆராய்ச்சியின் குறிக்கோள்

முதன்மைஆய்வாளரின் பெயர்: ரமீஸுதீன்

கைப்பேசி: 9677005828

இந்ததகவல்தாளின் உள்ளடக்கங்களை நான் கவனமாக படித்தேன் / அது என்னுடைய மொழியில் எனக்கு விளக்கப்பட்டது. நான் அவற்றை முழுமையாகப் புரிந்து கொண்டேன். எனக்கு எற்பட்ட சந்தேகங்களை தீர்த்துகொள்ள வாய்ப்பு அளிக்கப்பட்டது என்பதை உறுதிப்படுத்துகிறேன்.

இந்தஆராய்ச்சியின் முடிவில் என்னுடைய பெயர் மற்றும் என்னை பற்றிய தகவல்கள் வெளியே வராது என்பதையும், நான் விரும்பும் பட்சத்தில் இவ்வாராய்ச்சியிலிருந்து எப்பொழுது வேண்டுமானாலும் விலகி கொள்ளலாம் என்றும் அவ்வாறு விலகும் பொழுது அது எனக்கு அளிக்கப்படும் சிகிச்சையை ஒருபொழுதும் பாதிக்காது என்றும் அறிந்து கொண்டேன்.

இந்தஆராய்ச்சியில் பங்கேற்பதற்கு முழுமனதாக நான் சம்மதிக்கின்றேன்.

நோயாளியின் கையொப்பம் / இடது பெருவிரல் ரேகை

ஆராய்ச்சியாளரின் கையொப்பம்

பங்கேற்பவரின் பெயர்: _____

பங்கேற்பவரின் முகவரி:

_____

_____

_____

சாட்சி(1)

பெயர்:

முகவரி::

கையொப்பம்:

சாட்சி(2)

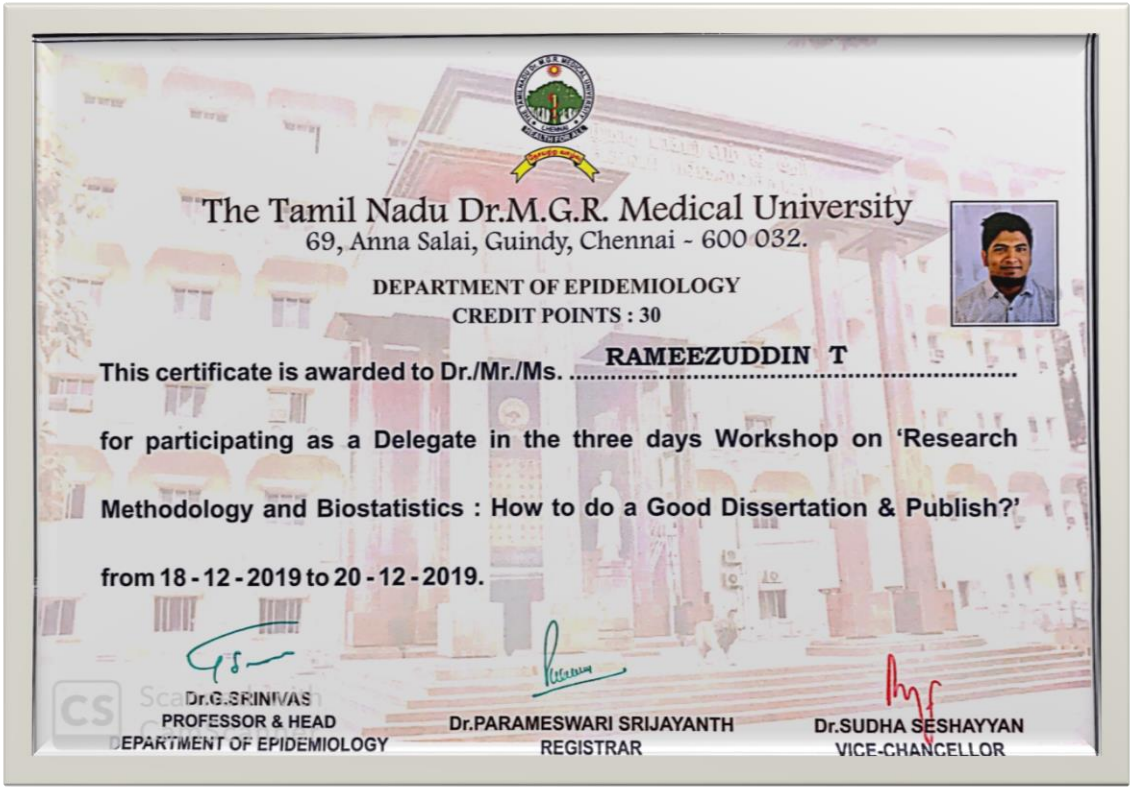
பெயர்:

முகவரி:

கையொப்பம்:

ANNEXURE V

RESEARCH METHODOLOGY CERTIFICATE



The Tamil Nadu Dr.M.G.R. Medical University
69, Anna Salai, Guindy, Chennai - 600 032.

DEPARTMENT OF EPIDEMIOLOGY
CREDIT POINTS : 30

This certificate is awarded to Dr./Mr./Ms. RAMEEZUDDIN T

for participating as a Delegate in the three days Workshop on 'Research Methodology and Biostatistics : How to do a Good Dissertation & Publish?'

from 18 - 12 - 2019 to 20 - 12 - 2019.

Dr.G.SRINIVAS
PROFESSOR & HEAD
DEPARTMENT OF EPIDEMIOLOGY

Dr.PARAMESWARI SRIJAYANTH
REGISTRAR

Dr.SUDHA SESHAYYAN
VICE-CHANCELLOR